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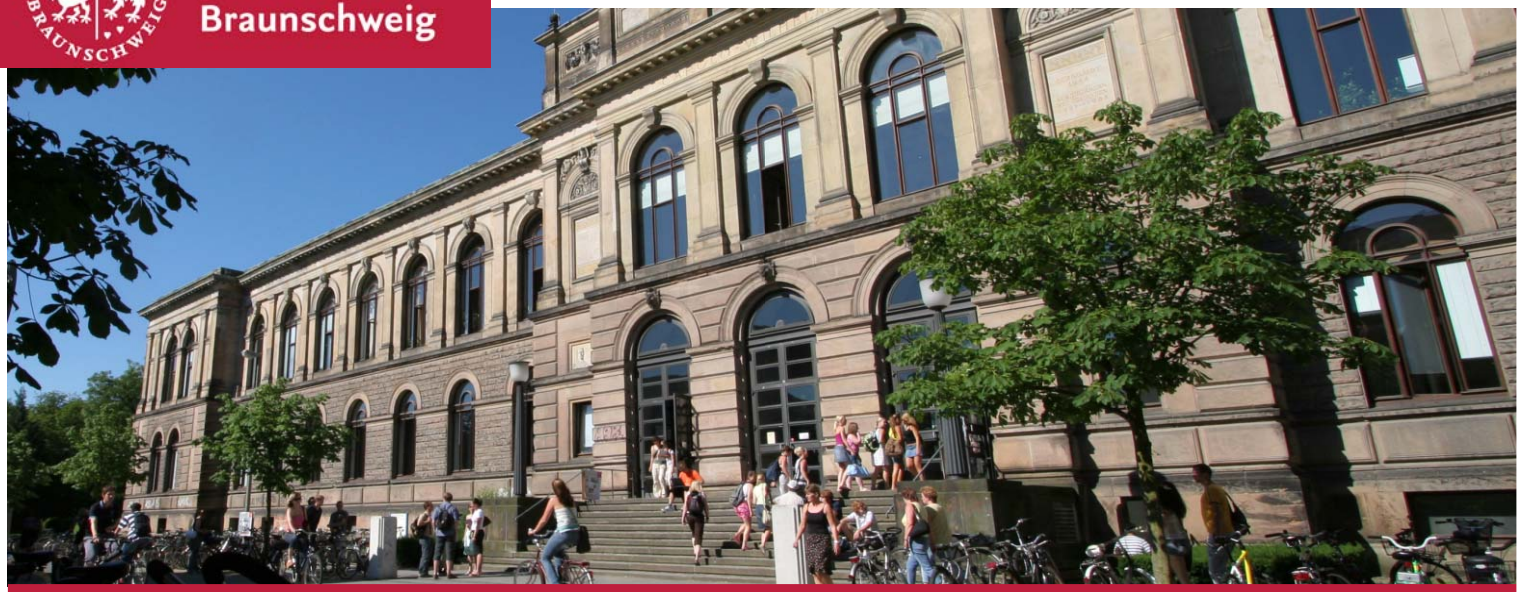

DPhG

Jahrestagung 2010

Personalisierte Therapeutika – Traum oder Wirklichkeit?

4. - 7. Oktober 2010

Tagungsband

Pharmazie in Braunschweig

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Jahrestagung 2010

Programm
&
Tagungsband

Institute der Pharmazie
Technische Universität Carolo-Wilhelmina zu Braunschweig

4.-7. Oktober

Personalisierte Therapeutika – Traum oder Wirklichkeit?

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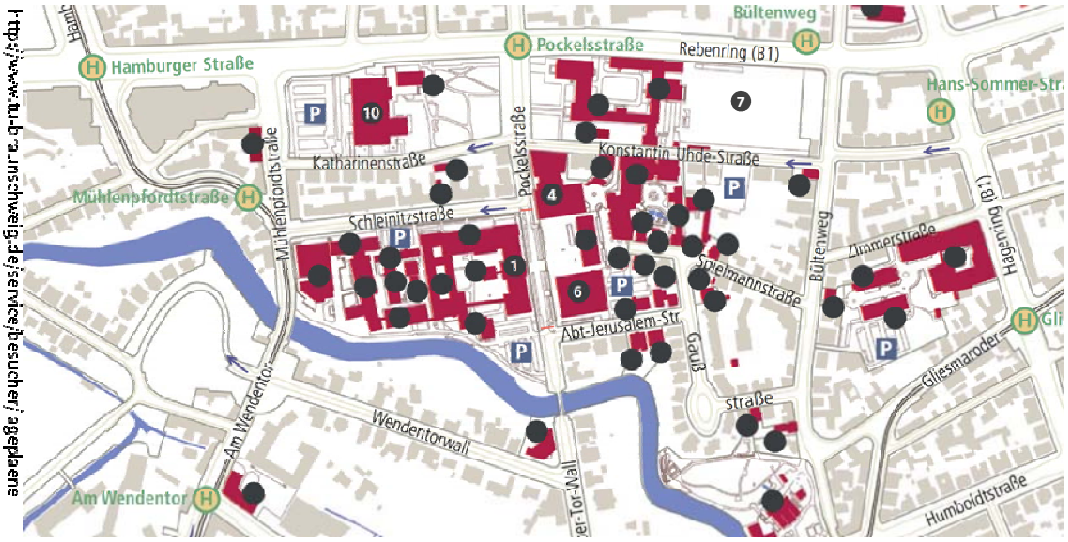
Lagepläne

Lageplan – Hauptgebäude und Audimax



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Haupttagung
Pharmaziehistorische Veranstaltung
Fachgruppensymposien
Tagungsbüro

6/7 Audimax/Tentomax
Eröffnung
Verabschiedung

4 Uni-Bibliothek
Ausstellung

10 Mensa

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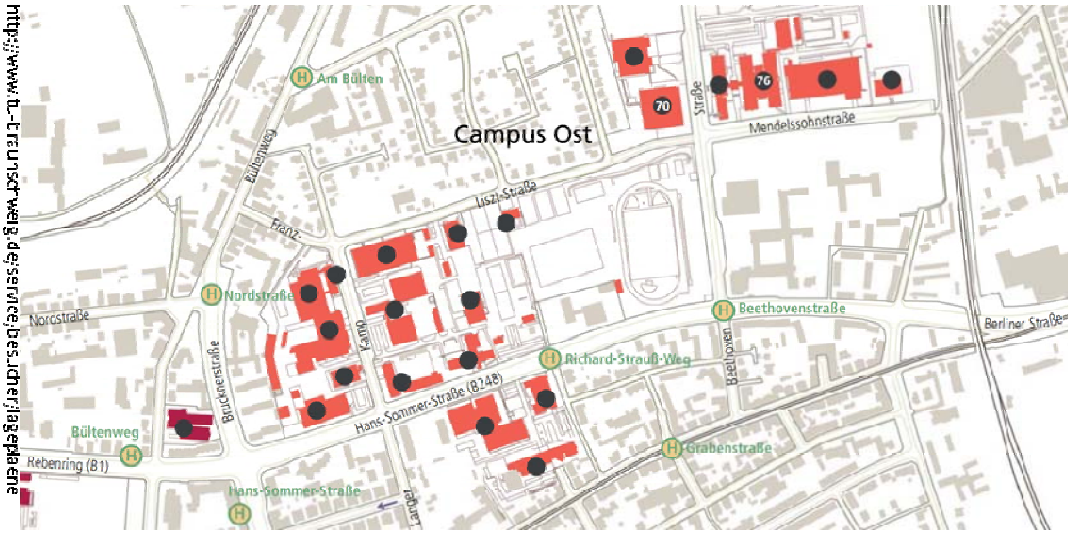

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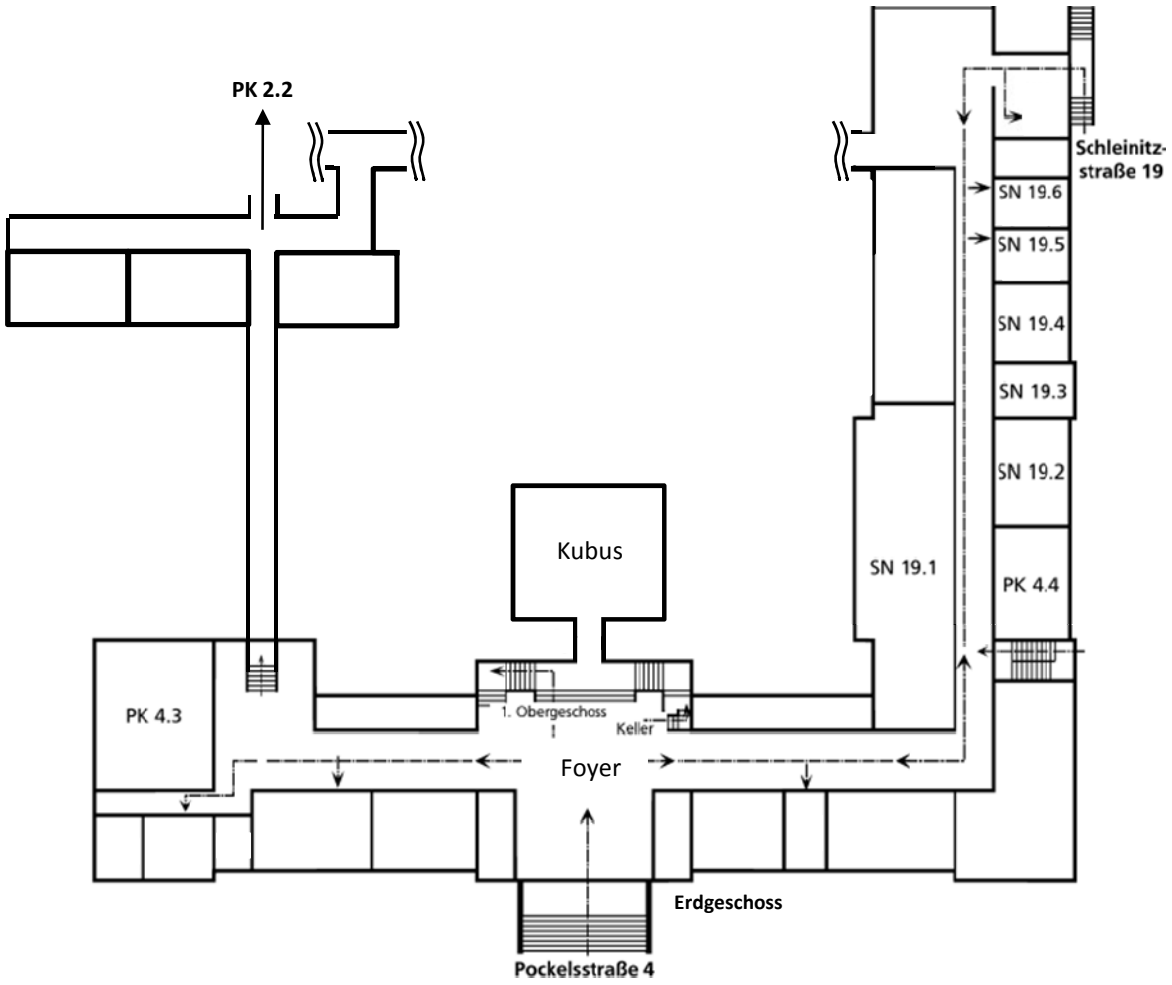
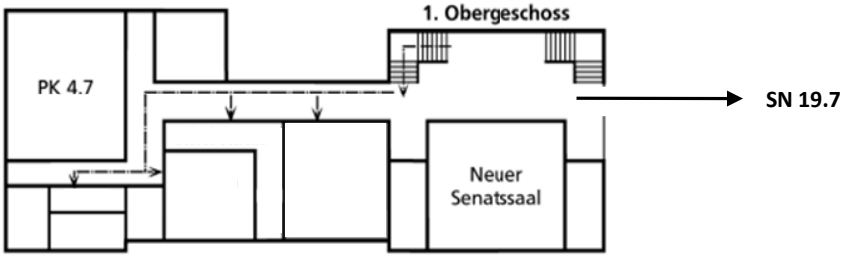
Lageplan – Hauptgebäude





Programm

Eröffnung der DPhG-Jahrestagung 2010 in Braunschweig

Montag, 04. Oktober 2010
13.00 Uhr
Audimax/ Tentomax

Prof. Dr. Christel C. Müller-Goymann
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Deutsche Pharmazeutische Gesellschaft e.V.

Montag, 4. Oktober 2010

13:00 - 13:30	Eröffnung (Audimax/ Tentomax)	
13:30 - 14:15	Plenarvortrag From Systems Biology to Personalized Medicine - Vision, wishful thinking or just a hype? (Audimax/ Tentomax)	
14:15 - 16:00	Ausstellung (Kubus)/ Posterpräsentation (Foyer)/ Kaffeepause (Kubus & Foyer)	
16:00 - 16:45	Plenarvorträge The practice of metabonomics/ metabolomics in the search for biomarkers (SN 19.1)	Ausgeträumt - Infektionskrankheiten, ihr Verschwinden und ihre Rückkehr im 20. Jahrhundert (PK 2.2)
16:50 - 17:50	Keynotes • Mikrosysteme für partikuläre Life Science Produkte (PK 4.7) • Regulatory RNAs - from identification to therapeutic application (PK 4.3)	Kurzvorträge • Klinische Pharmazie (SN 19.4) • Phytopharmaka (SN 19.3)
17:50 - 18:15	Kaffeepause (Foyer & Kubus)	
18:15 - 19:15	Keynotes • Computational Drug Design (PK 4.3)	Kurzvorträge • Biologisch aktive Naturstoffe (SN 19.3) • Hormone (SN 19.4) • Mikrosysteme für partikuläre Life Science Produkte (PK 4.7)
19:00 - 20:00	Podiumsdiskussion Arzneimittelfälschungen und Arzneimittelsicherheit (SN 19.2)	
ab 19:30	Begrüßungsabend (Foyer & Kubus)	

Montag, 4. Oktober 2010

Plenarvorträge

13:30 Balling, R. Pl-01
FROM SYSTEMS BIOLOGY TO PERSONALIZED MEDICINE – VISION, WISHFUL THINKING OR JUST A HYPE?
(Audimax/ Tentomax)
Moderation: Christel C. Müller-Goymann, Technische Universität Braunschweig

16:00 Wilson, I.D. Pl-02
THE PRACTICE OF METABONOMICS/METABOLOMICS IN THE SEARCH FOR BIOMARKERS
(SN 19.1)
Moderation: Hermann Wätzig, Technische Universität Braunschweig

16:00 Gradmann, C. Pl-03
AUSGETRÄUMT – INFEKTIONSKRANKHEITEN, IHR VERSCHWINDEN UND IHRE RÜCKKEHR IM 20. JAHRHUNDERT
(PK 2.2)
Moderation: Bettina Wahrig, Technische Universität Braunschweig

Podiumsdiskussion

19:00 Fink, E.; Bejeuhr, G.; Cramer, M.; Holzgrabe, U.
WIE ERKENNE ICH EINE ARZNEIMITTELFÄLSCHUNG?
(SN 19.2)

Montag, 4. Oktober 2010

Keynotes

Mikrosysteme für partikuläre Life Science Produkte (PK 4.7)
Moderation: Claus-Peter Klages, Technische Universität Braunschweig

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17:20	Krull, R.; Edlich, A.; Demming, S.; Zadeh, S.; Radespiel, R.; Büttgenbach, S.; Franco-Lara, E. MICROBIOREACTORS – A SCREENING-TOOL FOR BIOLOGICAL PROCESSES	Key1-2
17:35	Bunjes, H.; Fehr, S.; Finke, J.H.; Schur, J.; Müller-Goymann, C.C.; Lesche, C.; Büttgenbach, S.; Gothsch, T.; Kwade, A.; Jasch, K.; Huzhalska, V.; Kulik, A.; Augustin, W.; Scholl, S. PREPARATION OF LIPID NANOPARTICLES IN MICRO-STRUCTURED SYSTEMS	Key1-3

Regulatory RNAs - from identification to therapeutic application (PK 4.3)
Moderation: Roland K. Hartmann, Philipps-Universität Marburg

16:50	Jäschke, A.; Samanta, A.; Strauß, B.; Winz, M. CHEMICAL RNOMICS - THE SEARCH FOR NEW REGULATORY RNAS	Key2-1
17:10	Helm, M.; Hirsch, M. FLUORESCENCE SPECTROSCOPY BASED ANALYSIS OF SMALL INTERFERING RNA INTEGRITY DURING FORMULATION, TRANSFECION, AND INTRACELLULAR DISTRIBUTION	Key2-2
17:30	Hartmann, R.K.; Thomas, M.; Lange-Grünweller, K.; Weirauch, U.; Gutsch, D.; Aigner, A.; Grünweller, A. REPRESSION OF THE PROTO-ONCOGENE PIM-1 BY MIR-33A	Key2-3

Computational Drug Design (PK 4.3)
Moderation: Knut Baumann, Technische Universität Braunschweig

18:15	Meier, R.; Pippel, M.; Baldauf, C.; Sippl, W. PARADOCKS - A FRAMEWORK FOR MOLECULAR DOCKING WITH POPULATION-BASED METAHEURISTICS	Key3-1
18:35	Scheiber, J. SEEING THE WOOD, NOT ONLY THE TREES - SYSTEMS CHEMICAL BIOLOGY	Key3-2
18:55	Wolber, G. PHARMACOPHORE-BASED VIRTUAL SCREENING: AN EFFICIENT TOOL FOR BIO-ACTIVITY PROFILING AND AFFINITY PREDICTION	Key3-3

Montag, 4. Oktober 2010

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Klinische Pharmazie (SN 19.4)
Moderation: Ralf Benndorf, Technische Universität Braunschweig

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17:05 Niemann, D.; Ewen, A.L.; Oelsner, S.; Köpf, E.; Traiser, C.; Seebald, K.; Henhapl, T.; Meyburg, J.; Ruef, P.; Schmitt, C.P.; Bertsche, A.; Haefeli, W.E.; Bertsche, T. K1-2
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17:20 Niebecker, R.; Kuester, K.; Kunz, U.; Kloft, C. K1-3
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17:35 Birkle, S.; Schlager, H.; Dörje, F.; Lee, G.; Richter, W. K1-4
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Moderation: Christian Fleck, Friedrich-Schiller-Universität Jena

16:50 Abdel-Aziz, H.; Wadie, W.; Kelber, O.; Weiser, D.; Khayyal, M.T. P1-1
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17:05 Klein, K.; Merkel, K.; Jandaghi, D.; Kelber, O.; Vinson, B.R.; Weiser, D.; Klessen, C.; Rammensee, H.G.; Heinle, H. P1-2
LOCALISATION AND PHARMACOLOGY OF HISTAMINE-INDUCED FREE RADICAL PRODUCTION IN SMALL AND LARGE INTESTINE

17:20 Kelber, O.; Bonaterra, G.A.; Zügel, S.; Hildebrandt, W.; Weiser, D.; Kinscherf, R. P1-3
ANTI-PROLIFERATIVE EFFECTS OF THE ANTIDYSPEPTIC DRUG STW 5 IN COMPARISON WITH NSAIDS

17:35 Unger, M.; Völker, M.; Schaeflein, L.; Frank, A. P1-4
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Biologisch aktive Naturstoffe (SN 19.3)
Moderation: Ludger Beerhues, Technische Universität Braunschweig

18:15 Mundt, S.; Bui, H.; Le, T.; Zainuddin, E.; Jansen, R.; Nimtz, M.; Wray, V.; Preisitsch, M. B1-1
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Montag, 4. Oktober 2010

18:30	Probst, K.; Bechthold, A. CHANGING A MUTANT'S MIND	B1-2
18:45	Kaufmann, D.; Kaur Dogra, A.; Tahrani, A.; Herrmann, F.; Wink, M. TRADITIONAL CHINESE MEDICAL PLANTS INHIBIT ACETYL-CHOLINESTERASE, A KNOWN ALZHEIMER TARGET	B1-3
19:00	Belkheir, A.; Hänsch, R.; Beerhues, L. IMMUNOFLUORESCENCE LOCALIZATION OF POLYKETIDE SYNTHASES IN THE MEDICINAL PLANT *HYPERICUM PERFORATUM*	B1-4

Hormone (SN 19.4)
Moderation: Klaus Mohr, Rheinische Friedrich-Wilhelm-Universität Bonn

18:15	Hatlapatka, K.; Matz, M.; Baumann, K.; Rustenbeck, I. HOW DOES THE INSULIN GRANULE BEHAVE BEFORE ITS RELEASE? - TIRF MICROSCOPY ANALYSIS	P2-1
18:30	Matz, M.; Hatlapatka, K.; Rustenbeck, I.; Baumann, K. TOWARDS FULLY AUTOMATED TIRF MICROSCOPY IMAGE DATA ANALYSIS	P2-2
18:45	Dehm, F.; Pergola, C.; Jazzar, B.; Rossi, A.; Laufer, S.; Sautebin, L.; Werz, O. SEX BIAS IN LEUKOTRIENE GENERATION CAUSES GENDER-SPECIFIC EFFICACY OF LEUKOTRIENE SYNTHESIS INHIBITORS	P2-3
19:00	Rogge, A.; Pergola, C.; Werz, O. INFLUENCE OF PREGNANCY ON LEUKOTRIENE FORMATION: A PRIME EXAMPLE FOR PERSONALIZED MEDICINE	P2-4

Mikrosysteme für partikuläre Life Science Produkte (PK 4.7)
Moderation: Rolf Schubert, Albert-Ludwigs-Universität Freiburg

18:15	Lesche, C.; Holle, A.; Finke, J.H.; Müller-Goymann, C.C.; Büttgenbach, S. EMULSIFICATION (O/W) IN MICROCHANNEL GEOMETRIES FOR PHARMACEUTICAL SCREENING APPLICATIONS	T1-1
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18:45	Kähler, C.J.; Cierpka, C.; Segura, R.; Rossi, M. 3D FLOW FIELD MEASUREMENTS IN COMPLEX MICROSYSTEMS	T1-3
19:00	Schmolke, H.; Finke, J.H.; Müller-Goymann, C.C.; Klages, C.P. ADHESION OF SOLID LIPID NANOPARTICLES ON POLYELECTROLYTE MULTILAYER COATED SURFACES	T1-4

Dienstag, 5. Oktober 2010

09:00 - 09:45	**Plenarvorträge** Möglichkeiten und Grenzen individualisierter Medizin (SN 19.1)	**Plenarvorträge** Bacterial infections at atomic resolutions (PK 2.2)
09:50 - 10:50	**Keynotes** • Biotechnology of bioactive compounds from plants (SN 19.4) • Therapieindividualisierung in der Onkologie: Klinische Anwendungen und Trends in der Forschung (SN 19.2)	**Kurzvorträge** • Biochemie/Molekularbiologie (SN 19.7) • Nanopartikel (PK 4.7) • Pharmakokinetik (SN 19.3) • Wirkstoffsynthese (PK 4.3)
10:50 - 11:15	Kaffeepause (Foyer & Kubus)	
11:15 - 12:00	**Plenarvorträge** The genome as a tool for clinical pharmacy (SN 19.1)	**Plenarvorträge** Colloids as vaccine delivery systems - Kolloide als Impfstoffträger (PK 2.2)
12:00 - 13:30	Mittagspause/ VdPPHI-Sitzung (SN 19.2)	
13:30 - 14:15	**Plenarvorträge** Die Bedeutung von Stammzellen für die Diabetes-Therapie (SN 19.1)	**Plenarvorträge** Pharmacological inhibitors of cyclin-dependent protein kinases relevant to cancer (PK 2.2)
14:15 - 16:15	Ausstellung (Kubus)/ Posterpräsentation (Foyer)/ Kaffeepause (Kubus & Foyer)	
16:15 - 17:00	**Plenarvorträge** Entwicklung neuer antitumoraler Metallkomplexe (SN 19.1)	**Plenarvorträge** Hyperforin - From the herb to the molecule and target (PK 2.2)
17:00 - 17:15	Kaffeepause (Foyer & Kubus)	
17:15 - 18:15	**Keynotes** • Enzyme in der Wirkstofffindung (SN 19.2)	**Kurzvorträge** • Analytik (PK 4.3) • Antitumorwirkstoffe (SN 19.7) • Polymere für die Implantation (PK 4.7) • Signaltransduktion (SN 19.3)
ab 19:30	Gesellschaftsabend begleitet von Jazz Appeal (Mensa II)	

Dienstag, 5. Oktober 2010

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BACTERIAL INFECTIONS AT ATOMIC RESOLUTION
(PK 2.2)
Moderation: Ute Wittstock, Technische Universität Braunschweig

09:00 Kroemer, H.K. Pl-05
MÖGLICHKEITEN UND GRENZEN INDIVIDUALISIERTER MEDIZIN
(SN 19.1)
Moderation: Ralf Benndorf, Technische Universität Braunschweig

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Moderation: Heike Bunjes, Technische Universität Braunschweig

11:15 McLeod, H.L. Pl-07
THE GENOME AS A TOOL FOR CLINICAL PHARMACY
(SN 19.1)
Moderation: Conrad Kunick, Technische Universität Braunschweig

13:30 Seufert, J. Pl-08
DIE BEDEUTUNG VON STAMMZELLEN FÜR DIE DIABETES-THERAPIE
(SN 19.1)
Moderation: Ingo Rustenbeck, Technische Universität Braunschweig

13:30 Meijer, L. Pl-09
PHARMACOLOGICAL INHIBITORS OF CYCLIN-DEPENDENT PROTEIN KINASES RELEVANT TO CANCER
(PK 2.2)
Moderation: Conrad Kunick, Technische Universität Braunschweig

Dienstag, 5. Oktober 2010

16:15 Müller, W.E.; Leuner, K. Pl-10
HYPERFORIN – FROM THE HERB TO THE MOLECULE AND TARGET
(PK 2.2)
Moderation: Ludger Beerhues, Technische Universität Braunschweig

16:15 Keppler, B.K. Pl-11
ENTWICKLUNG NEUER ANTITUMORALER METALLKOMPLEXE
(SN 19.1)
Moderation: Ingo Ott, Technische Universität Braunschweig

Dienstag, 5. Oktober 2010

Keynotes

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Moderation: Ute Wittstock, Technische Universität Braunschweig

09:50	Halkier, B.A. BIOENGINEERING OF GLUCORAPHANIN FROM BROCCOLI	Key4-1
10:20	Liu, B.Y.; Wang, H.; Du, Z.G.; Li, G.F.; Ye, H.C. ENGINEERING *ARTEMISIA ANNUA* FOR ARTEMISININ PRODUCTION	Key4-2
10:35	Heckenmüller, H.; Selge, T.; Wilke, S.; Schütte, K.; Gorr, G. LONG-TERM STORAGE OF UNDIFFERENTIATED PLANT CELLS FOR PRODUCTION OF HIGH VALUE SUBSTANCES	Key4-3

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Moderation: Ralf Benndorf, Technische Universität Braunschweig

09:50	Hempel, G. INDIVIDUALIZATION OF ANTICANCER DRUG DOSING BASED ON PHARMACOKINETIC PRINCIPLES	Key5-1
10:10	Ritter, C.A. INDIVIDUALIZATION OF ANTICANCER DRUG TREATEMENT BASED ON PHARMACOGENETIC FACTORS	Key5-2
10:30	Jaehde, U. INDIVIDUALIZATION OF ANTICANCER DRUG THERAPY USING BIOMARKERS	Key5-3

Enzyme in der Wirkstofffindung (SN 19.2)
Moderation: Christa E. Müller, Rheinische Friedrich-Wilhelm-Universität Bonn

17:15	Rauh, D. STABILIZING INACTIVE KINASE CONFORMATIONS WITH SMALL ORGANIC MOLECULES	Key6-1
17:35	Beerhues, L. PLANT POLYKETIDE SYNTHASES IN THE BIOSYNTHESIS OF ACTIVE CONSTITUENTS	Key6-2
17:55	Müller, M. CHEMOENZYMATISCHE WIRKSTOFF-SYNTHESE	Key6-3

Dienstag, 5. Oktober 2010

Kurzvorträge

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Moderation: Joachim José, Heinrich-Heine Universität Düsseldorf

09:50 Erdmann, N.; Folz, M.; Dobner, B.; Langner, A. C2-1
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10:05 Giera, M.; de Vlieger, J.; Falck, D.; Lingeman, H.; Kool, J.; Irth, H.; Niessen, W.M.A. C2-2
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10:20 Hartung, A.; Schlesinger, M.; Massing, U.; Bendas, G. C2-3
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10:35 Oehmigen, K.; Hähnel, M.; Hoder, T.; Wilke, C.; Weltmann, K.D.; von Woedtke, T. C2-4
PLASMA-LIQUID-INTERACTIONS: CHEMISTRY AND ANTIMICROBIAL EFFECTS

Nanopartikel (PK 4.7)
Moderation: Klaus Langer, Westfälische Wilhelms-Universität Münster

09:50 Müller, A.; Ni, Z.; Heßler, N.; Kralisch, D.; Fischer, D. T2-1
BACTERIAL NANOCELLULOSE: INFLUENCE OF FREEZE-DRYING ON THE DELIVERY OF DRUGS

10:05 Möschwitzer, J. T2-2
DRUG NANOPARTICLES PREPARED BY NOVEL COMBINATIVE PARTICLE SIZE REDUCTION TECHNIQUES

10:20 Hozsa, C.; Breunig, M.; Göpferich, A. T2-3
SHEDDING LIGHT ON THE INTRACELLULAR PROCESSING OF REDUCTION SENSITIVE POLY(ETHYLENE IMINE) GENE CARRIERS

10:35 Nawroth, T.; Wurster, E.C.; Peters, T.; Buch, K.; Huehn, E.; Langguth, P.; Decker, H.; Pairet, B.; Meesters, C.; Grunewald, C.; Hampel, G.; Frey, H.; Hofmann, A.M.; Schmidberger, H.; Saenger, M.; Alexiou, C. T2-4
MODULAR TARGET NANOPARTICLES – DRUG CARRIERS FOR RADIATION THERAPY OF CANCER

Pharmakokinetik (SN 19.3)
Moderation: Thilo Bertsche, UniversitätsKlinikum Heidelberg

09:50 Oswald, S.; Meyer zu Schwabedissen, H.; Nassif, A.; Modess, C.; Lütjohann, D.; Desta, Z.; Kroemer, H.K.; Siegmund, W. P3-1
IMPACT OF EFAVIRENZ ON INTESTINAL AND HEPATIC METABOLISM AND TRANSPORT: INTERACTION STUDY WITH EZETIMIBE IN HEALTHY VOLUNTEERS

Dienstag, 5. Oktober 2010

10:05 Peters, J.; Oswald, S.; Haenisch, S.; Ludwig, K.; Bernhardt, J.; Saljé, K.; Modess, C.; Cascorbi, I.; Siegmund, W. P3-2
INFLUENCE OF ROUX-EN-Y GASTRIC BYPASS SURGERY ON THE PHARMACOKINETICS OF PARACETAMOL, TALINOLOL AND AMOXICILLIN IN OBESE PATIENTS

10:20 Parr, M.K.; Diel, P.; Schänzer, W. P3-3
THE SARM-LIKE ACTIVITY OF SUPPLEMENT INGREDIENT NOR-ANDROSTENEDIONE DEPENDS ON ROUTE OF ADMINISTRATION

10:35 Weindl, G.; Klipper, W.; Bätz, F.M.; Schäfer-Korting, M. P3-4
COMPARATIVE ANALYSIS OF ESTERASE ACTIVITY IN RECONSTRUCTED HUMAN SKIN MODELS AND EXCISED HUMAN SKIN

Wirkstoffsynthese (PK 4.3)

Moderation: Peter Gmeiner, Friedrich-Alexander-Universität Erlangen-Nürnberg

09:50 Bracher, F.; Huber, K.; Knapp, S. C1-1
4-CYANO-1-OXO-β-CARBOLINES AS INHIBITORS OF PIM KINASES

10:05 Behrendt, C.T.; Eisenreich, W.; Fischer, M.; Maes, L.; Kurz, T. C1-2
SYNTHESIS AND ANTIPLASMODIAL ACTIVITY OF REVERSE FOSMIDOMYCIN ANALOGS

10:20 Dosa, S.; Stirnberg, M.; Klaß, V.; Häußler, D.; Maurer, E.; Gütschow, M. C1-3
SULFAMOYL BENZAMIDINES AS ARGININE MIMETICS: INHIBITION OF TRYPSIN-LIKE SERINE PROTEASES AND ACTIVE-SITE MAPPING

10:35 Meyer, C.; Wünsch, B. C1-4
SPIROCYCLIC σ RECEPTOR LIGANDS: EXPLORING HYDROPHOBIC POCKETS BY ARLYATION OF ANNULATED THIOPHENES

Analytik (PK 4.3)

Moderation: Thomas Jira, Ernst-Moritz-Arndt Universität Greifswald

17:15 Alban, S.; Schiemann, S.; Lühn, S.; Schneider, T. C3-1
COMPREHENSIVE QUALITIY CONTROL OF HEPARINS BY A SIMPLE MICROPLATE ASSAY PROCEDURE

17:30 Lalk, M.; Dörries, K.; Gierok, P.; Liebeke, M.; Meyer, H.; Wunder, A. C3-2
A METABOLOMICS VIEW ON STAPHYLOCOCCUS AUREUS

17:45 Bertram, N.; Ostermeyer, M.; Gottsleben, F. C3-3
NEW INSIGHTS WITH 'OLD' METHODS? HIGH PRECISION POLARIMETRY AND REFRACTOMETRY: FROM KAISER'S GELATINE TO DETECTING BIOLOGICAL WARFARE AGENTS

18:00 Pettelkau, J.; Ihling, C.; Schröder, T.; Olausson, B.; Lange, C.; Sinz, A. C3-4
INTERACTION STUDIES BETWEEN PEPTIDES DERIVED FROM PHOTORECEPTOR GUANYLYL CYCLASE AND GCAP-2

Dienstag, 5. Oktober 2010

Antitumorwirkstoffe (SN 19.7)
Moderation: Sigurd Elz, Universität Regensburg

17:15	Krieger, M.L.; Schneider, V.; Kalayda, G.V.; Jaehde, U.; Bendas, G. CISPLATIN-CONTAINING LIPOSOMES TO INVESTIGATE THE MECHANISMS OF CHEMORESISTANCE IN TUMOUR CELLS	C4-1
17:30	Oster, A.; Hinsberger, S.; Werth, R.; Marchais-Oberwinkler, S.; Frotscher, M.; Hartmann, R.W. NEW DESIGN CONCEPT FOR THE DEVELOPMENT OF 17β-HSD1 INHIBITORS: PROMISING DRUG CANDIDATES FOR THE TREATMENT OF ESTROGEN DEPENDENT DISEASES	C4-2
17:45	Westendorf, A.F.; Zerzankova, L.; Grünert, R.; Sadler, P.J.; Brabec, V.; Bednarski, P.J. LIGHT-ACTIVATABLE TRANS-DIAZIDO PT(IV): BIOLOGICAL ACTIVITY AND THE INFLUENCE OF AMINO LIGANDS	C4-3
18:00	Tolle, N.; Dunkel, U.; Müller, C.; Preu, L.; Oehninger, L.; Rubbiani, R.; Meyer, A.; Ott, I.; Haase, T.; Behrends, S.; Totzke, F.; Schächtele, C.; Kubbutat, M.H.G.; Kunick, C. NOVEL FLUORESCENT PROTEIN KINASE INHIBITORS	C4-4

Polymere für die Implantation (PK 4.7)
Moderation: Dagmar Fischer, Friedrich-Schiller-Universität Jena

17:15	Asmus, L.R.; Gurny, R.; Möller, M. A POLYMER AS SOLVENT AND SUSTAINED RELEASE EXCIPIENT FOR LIPOPHILIC DRUGS – HEXYLSUBSTITUTED POLY(LACTIDE)	T3-1
17:30	Dempwolf, W.; Pfaffenroth, C.; Sluszniak, M.; Lorenz, C.; Hoffmann, A.; Winkel, A.; Stiesch, M.; Windhagen, H.; Menzel, H. DESIGNING POLYMER INTERLAYERS TO IMPROVE IMPLANT SURFACES	T3-2
17:45	Nowak, C.; Metz, H.; Mäder, K.; Hacker, M.; Schulz-Siegmund, M. VEGF RELEASE FROM CA-/ZN-ALGINATE GELS AND THEIR PHYSICO CHEMICAL PROPERTIES	T3-3
18:00	Teßmar, J.; Reintjes, T.; Göpferich, A. OPTIMIZED DEGRADATION AND MECHANICAL PROPERTIES OF POLYMER FILMS FOR SURGICAL ADHESION PREVENTION	T3-4

Signaltransduktion (SN 19.3)
Moderation: Angelika M. Vollmar, Ludwig-Maximilians-Universität München

17:15	Janßen, N.; Kebig, A.; Kostenis, E.; Mohr, K. THE Gq-COUPLED MUSCARINIC M_3 RECEPTOR GAINS Gi SIGNALING COMPETENCE UNDER CONDITIONS OF ENHANCED cAMP	P4-1
17:30	Bock, A.; Holzgrabe, U.; De Amici, M.; Mohr, K. LINKER LENGTH IS PIVOTAL FOR POTENCY OF DUALSTERIC AGONISTS AT MUSCARINIC M_2 RECEPTORS	P4-2

Dienstag, 5. Oktober 2010

17:45 Michaelis, M.; Paulus, C.; Löschmann, N.; Dauth, S.; Stange, E.; Doerr, H.W.; Nevels, M.; Cinatl, J. P4-3
THE MULTI-TARGETED KINASE INHIBITOR SORAFENIB INHIBITS HUMAN CYTOMEGALOVIRUS REPLICATION

Mittwoch, 6. Oktober 2010

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<tr><td>09:15 - 10:00</td><td colspan="2"><u>Plenarvorträge</u></td></tr>
<tr><td></td><td>Stimulators and activators of soluble guanylate cyclase: from bench to bedside (SN 19.1)</td><td>Polymers for the control of cell material interactions on the micro- and nanoscale (PK 2.2)</td></tr>
<tr><td>10:05 - 11:05</td><td><u>Keynotes</u>
Pharmakologische Wirkstoffoptimierung durch Polymerkonjugation (SN 19.2)</td><td><u>Kurzvorträge</u>
Analytik (PK 4.3)
Computerunterstützte Wirkstofffindung (SN 19.4)
Dermale Therapie (PK 4.7)
Feste Arzneiformen (SN 19.7)
Herz-Kreislauf (SN 19.3)</td></tr>
<tr><td>11:05 - 11:30</td><td colspan="2">Kaffeepause (Foyer & Kubus)</td></tr>
<tr><td>11.30 - 12:15</td><td colspan="2"><u>Plenarvortrag</u>

Evidence-based complementary medicine - a contradiction in terms?
(Audimax/ Tentomax)</td></tr>
<tr><td>12:15 - 13:00</td><td colspan="2">Preisverleihungen, Ehrungen (Audimax/ Tentomax)</td></tr>
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Mittwoch, 6. Oktober 2010

Plenarvorträge

09:15 Stasch, J.P. Pl-12
STIMULATORS AND ACTIVATORS OF SOLUBLE GUANYLATE CYCLASE: FROM BENCH TO BEDSIDE
(SN 19.1)
Moderation: Soenke Behrends, Technische Universität Braunschweig

09:15 Brandl, F.; Teßmar, J.; Breunig, M.; Göpferich, A. Pl-13
POLYMERS FOR THE CONTROL OF CELL MATERIAL INTERACTIONS ON THE MICRO- AND NANOSCALE
(PK 2.2)
Moderation: Christel C. Müller-Goymann, Technische Universität Braunschweig

11:30 Ernst, E. Pl-14
EVIDENCE-BASED COMPLEMENTARY MEDICINE – A CONTRADICTION IN TERMS?
(Audimax/ Tentomax)
Moderation: Ingo Rustenbeck, Technische Universität Braunschweig

Mittwoch, 6. Oktober 2010

Keynotes

Pharmakologische Wirkstoffoptimierung durch Polymerkonjugation (SN 19.2)
Moderation: Roland Frank, Helmholz Zentrum für Infektionsforschung Braunschweig

10:05	Vorstheim, P. BIG IS BEAUTIFUL – HESylation® AS AN EXAMPLE FOR DRUG-POLYMER CONJUGATES	Key7-1
10:35	Kontermann, R. NEUE STRATEGIEN ZUR VERLÄNGERUNG DER HALBWERTSZEIT REKOMBINANTER PROTEINE	Key7-2
10:50	Apeler, H. NEXT GENERATION SITE-SPECIFICALLY PEGYLATED FVIII FOR THE TREATMENT OF HEMOPHILIA A	Key7-3

Mittwoch, 6. Oktober 2010

Kurzvorträge

Analytik (PK 4.3)
Moderation: Ulrike Holzgrabe, Julius-Maximilians-Universität Würzburg

10:05	Sproß, J.; Sinz, A. IMMOBILIZED MONOLITHIC TRYPSIN REACTOR FOR APPLICATION IN PHARMACEUTICS AND PROTEOMICS	C5-1
10:20	Kammerer, B.; Kahlich, R.; Laufer, S. ACHIRAL–CHIRAL LC/LC–MS/MS COUPLING FOR DETERMINATION OF CHIRAL DISCRIMINATION EFFECTS IN DRUG METABOLISM	C5-2
10:35	Telsnig, D.; Kassarnig, V.; Kalcher, K.; Ortner, A. OPTIMIZATION AND APPLICATION OF PEA SEEDLING AMINE OXIDASE MODIFIED BIOSENSORS	C5-3
10:50	Tawab, M.; Werz, O.; Schubert-Zsilavecz, M. FILLING THE GAP BETWEEN PHARMACOLOGICAL TESTING AND *IN VIVO* FINDING ON THE EXAMPLE OF *BOSWELLIA SERRATA*	C5-4

Computerunterstütze Wirkstofffindung (SN 19.4)
Moderation: Gerhard Wolber, Freie Universität Berlin

10:05	Schiedel, A.C.; Seibt, B.F.; Sherbiny, F.F.; Maaß, A.; Müller, C.E. ROLE OF THE SECOND EXTRACELLULAR LOOP OF THE ADENOSINE A_{2B} RECEPTOR IN RECEPTOR ACTIVATION	C6-1
10:20	Strasser, A.; Wittmann, H.J. IN SILICO ANALYSIS OF THE HISTAPRODIFEN INDUCED ACTIVATION PATHWAY OF THE GUINEA-PIG H_1-RECEPTOR	C6-2
10:35	Negri, M.; Recanatini, M.; Hartmann, R.W. DYNAMIC MOTION INVESTIGATION OF 17β-HSD1 PROVIDES INSIGHTS IN ITS ENZYME KINETICS AND LIGAND BINDING	C6-3

Dermale Therapie (PK 4.7)
Moderation: Rolf Daniels, Eberhard-Karls Universität Tübingen

10:05	Keck, C.M. THE SILVER - NANOLIPID - COMPLEX (sNLC): IN VIVO EFFICACY	T4-1
10:20	Hahn, T.; Nägel, A.; Heisig, M.; Kostka, K.H.; Hansen, S.; Neumann, D.; Lehr, C.M.; Schäfer, U.F. FINITE DOSE SKIN PENETRATION - EXPERIMENT AND SIMULATION	T4-2
10:35	Lusiana; Müller-Goymann, C.C. THE PERMEATION STUDY OF TERBINAFINE HCl FROM POLOXAMER 407 BASED THERMOGELLING FORMULATIONS ACROSS ISOLATED HUMAN STRATUM CORNEUM	T4-3

Mittwoch, 6. Oktober 2010

10:50 Michaelis, M.; Leopold, C.S. T4-4
INFLUENCE OF IBUPROFEN CONTENT ON THE RHEOLOGICAL AND THERMAL BEHAVIOR OF AN ACRYLIC PRESSURE SENSITIVE ADHESIVE

Feste Arzneiformen (SN 19.7)
Moderation: Jörg Breitkreutz, Heinrich-Heine-Universität Düsseldorf

10:05 Kleinebudde, P.; Knop, K.; Müller, J. T5-1
END POINT CONTROL OF AN ACTIVE COATING PROCESS BY RAMAN SPECTROSCOPY

10:20 Reitz, E.; Thommes, M. T5-2
HOT MELT EXTRUSION OF LOW MOLECULAR WEIGHT CRYSTALLINE MATERIALS

10:35 Metzger, P.O.J.; Wahl, M.A. T5-3
POROUS CARRIERS AS A TARGET FOR DRUG LOADING BY SUPERCRITICAL FLUID TECHNOLOGY USING AN OPEN OR ENVIRONMENT FRIENDLY CLOSED LOOP SYSTEM

10:50 Taupitz, T.; Klein, S. T5-4
VARIOUS FORMULATION APPROACHES TO IMPROVE DRUG RELEASE FROM A FIXED DOSE COMBINATION PRODUCT

Herz-Kreislauf (SN 19.3)
Moderation: Christoph Ritter, Ernst-Moritz-Arndt-Universität Greifswald

10:05 Khayyal, M.T.; Abdel-Aziz, H.; El-Awady, S.; Ammar, R. P5-1
STUDIES ON THE MECHANISM OF ANTIHYPERTENSIVE ACTION OF SOLANUM INDICUM SSP. DISTICHUM

10:20 Krähling, J.R.; Busker, M.; Haase, N.; Haase, T.; Linnenbaum, M.; Oberle, S.; Behrends, S. P5-2
ANALYSIS OF REQUIREMENTS FOR DIMERIZATION OF NITIRIC OXIDE SENSITIVE GUANYLYL CYCLASE

10:35 Liebl, J.; Weitensteiner, S.B.; Vereb, G.; Takacs, L.; Fürst, R.; Vollmar, A.M.; Zahler, S. P5-3
CYCLIN DEPENDENT KINASE 5 (CDK5) REGULATES ENDOTHELIAL CELL MIGRATION AND ANGIOGENESIS

10:50 Fürst, R.; Schmerwitz, U.K.; Sass, G.; Khandoga, A.G.; Joore, J.; Totzke, F.; Krombach, F.; Tiegs, G.; Zahler, S.; Vollmar, A.M. P5-4
FLAVOPIRIDOL PROTECTS AGAINST INFLAMMATION BY INHIBITION OF CDK9

Pharmaziehistorische Veranstaltung

Pharmaziehistorische Veranstaltung

Pharmazie in Braunschweig: Historische und aktuelle Aspekte

(SN 19.4)

Montag, 04. Oktober, 2010

09:00	Begrüßung und Einführung Dilg, P.	
09:15	Beisswanger, G.; Wacker, G. HOF – STADT – LAND. APOTHEKEN IM HERZOGTUM BRAUNSCHWEIG-WOLFENBÜTTEL	H-1 (n.v.)
10:00	Wahrig, B. UNIVERSITÄTSPHARMAZIE IN BRAUNSCHWEIG VON 1835 BIS HEUTE	H-2 (n.v.)
10:45	Pause	
11:15	Pohl, U. FRIEDRICH JULIUS OTTO ZWISCHEN UNIVERSITÄT UND GEWERBE	H-3 (n.v.)
12:00	Wulle, S. DAS DFG-SONDERSAMMELGEBIET PHARMAZIE DER UB BRAUNSCHWEIG	H-4 (n.v.)
12:45	Ende der Veranstaltung	

Fachgruppensymposien

Fachsymposium Allgemeinpharmazie

Pharmazeutische Betreuung und Arzneimittelsicherheit

(SN 19.2)

Mittwoch, 06. Oktober, 2010

14:30 Themeneinführung und Vorstellung der Referenten
Hannig, M.; Kresser, J.

14:45 Aly, A.F. F1-1
FÖRDERUNG DER KOOPERATION VON ARZT UND APOTHEKER ALS THEMA/ MAßNAHME DES NEUEN AKTIONSPLANS DES BUNDESMINISTERIUMS FÜR GESUNDHEIT ZUR VERBESSERUNG DER ARZNEIMITTELTHERAPIESICHERHEIT (AMTS)

15:20 Schwenzer, S. F1-2
ZUKUNFT eMEDIKATION. WIE IT DIE PHARMAZEUTISCHE BETREUUNG UNTERSTÜTZEN KANN

15:55 Schäfer, M. F1-3
ERSCHLIEßUNG VON SICHERHEITS- UND WIRTSCHAFTLICHKEITSRESERVEN DURCH DIE DOKUMENTATION ARZNEIMITTELBEZOGENER PROBLEME

16:30 Podiumsdiskussion AMTS/ Pharmazeutische Betreuung/ Praxis und Zukunft
Schäfer, M.; Holzgrabe, U.; Aly, A.F.; Schwenzer, S.; Kresser, J.; Hannig, M.

17:30 Mitgliederversammlung der Fachgruppe Allgemeinpharmazie
- Bericht des Vorstands Dr. M. Hannig
- Neue Kommunikationswege der Fachgruppe
- Fachsymposium 2011
- Verschiedenes

Fachsymposium Arzneimittelkontrolle/ Pharmazeutische Analytik

Ein facettenreiches Spektrum: Pharmazeutische Analytik an Universitäten

(PK 4.3)

Mittwoch**,** 06. Oktober, 2010

14:30	Begrüßung	
14:45	Parr, M.K. TRACING CHEATERS IN SPORTS – MASS SPECTROMETRY IN ANTI-DOPING RESEARCH	F2-1
15:15	Holzgrabe, U. DAS ARZNEIBUCH UND ORTHOGONALE METHODEN	F2-2(n.v.)
15:45	Sproß, J.; Sinz, A. PREPARATION OF MONOLITHIC COLUMNS FOR LC-MS/MS ANALYSIS OF PROTEINS AND DRUGS	F2-3
16:15	Pause	
16:45	Scriba, G.K.E. CE IN PHARMACEUTICAL ANALYSIS – APPLICATION TO DRUG IMPURITY PROFILING	F2-4
17:15	Jose, J.; Gratz, A.; Götz, C. CAPILLARY ELECTROPHORESIS (CE) AND FRET AS TOOLS FOR TESTING INHIBITORS OF HUMAN PROTEINKINASE CK2	F2-5
17:45	Jung, M. AKTIVITÄTSASSAYS FÜR HISTON-MODIFIZIERENDE ENZYME IN WIRKSTOFFSUCHE UND BIOANALYTIK	F2-6(n.v.)
18:15	Pause	
18:30	Heilmann, J. NACHWEIS UND BESTIMMUNG VON PRENYLIERTEN CHALCONEN (XANTHOHUMOL)UND IHREN METABOLITEN IN ZELLKULTUREN, PLASMA UND GEWEBEN	F2-7(n.v.)
19:00	Speikamp, Feußner, Jäckel, Wätzig INDUSTRIEFORUM ANALYTIK: AKTUELLE AUSBILDUNGSINHALTE FÜR ZUKUNFTSWEISENDE KONZEPTE	F2-8(n.v.)
20:00	Gesellschaftsabend in der Braunschweiger Traditionskneipe „Mephisto“	

Fachsymposium Arzneimittelkontrolle/ Pharmazeutische Analytik

Physikalische und pharmazeutisch-technologische Methoden: State of the Art

(PK 4.3)

Donnerstag**,** 07. Oktober, 2010

08:45	Begrüßung	
09:00	Vielle, C. OVERVIEW ABOUT THE DEVELOPMENTS IN THE EUROPEAN PHARMACOPEIA IN TERMS OF PHYSICOCHEMICAL AND PHARMACEUTICAL TECHNICAL METHODS	F3-1(n.v.)
09:30	Langguth, P. BESTIMMUNG DER WIRKSTOFFFREISETZUNG IN-VITRO: METHODEN IM SPAGAT ZWISCHEN QUALITÄTSKONTROLLE UND BIORELEVANZ	F3-2(n.v.)
10:15	Roßricker, T. UNTERSUCHUNGSMETHODEN ZUR QUALITÄTSKONTROLLE VON AEROSOLEN	F3-3(n.v.)
10:45	Pause	
11:15	Wundrack, A. PH-MESSUNG IN KOMPLEXEN MATRICES	F3-4(n.v.)
11:45	Petrich, M. ANALYSE VON SPURENELEMENTEN IN PHARMAZEUTIKA MIT AAS, ICP-OES UND ICP-MS	F3-5(n.v.)
12:15	Raith, K. GALENISCHE UND PHYSIKALISCHE METHODEN IN DER AMTLICHEN ARZNEIMITTELUNTERSUCHUNG	F3-6(n.v.)
12:45	Abschlussdiskussion	

Fachsymposium Klinische Pharmazie

Arzneimittelsicherheit – Chancen für die Klinische Pharmazie

(SN 19.3)

Sonntag, 03. Oktober, 2010

14:00	Keiner, D. A YEAR CPOE - PRACTICAL EXPERIENCES AND PERSPECTIVES FROM A CLINICAL PHARMACIST	F4-1
14:45	Nowak, K. SAFETY OF PHARMACOTHERAPY ON THE INTERFACE BETWEEN OUTPATIENT AND INPATIENT TREATMENT	F4-2
15:30	Pause	
15:50	Bertsche, T. CLINICAL-PHARMACEUTICAL INTERVENTION STUDIES TO OPTIMISE PATIENT SAFETY IN DRUG THERAPY IN HOSPITAL SETTINGS	F4-3
16:35	Mahler, C. MEDICATION SAFETY – HOW DO NURSES CONTRIBUTE?	F4-4
17:20	Mitgliederversammlung der Fachgruppe Klinische Pharmazie	

Fachsymposium Klinische Pharmazie

Arzneimittelsicherheit – Chancen für die Klinische Pharmazie

(SN 19.3)

Montag, 04. Oktober, 2010

08:30	Jaehde, U.; Hanke, F. MEDICATION SAFETY OF ELDERLY PATIENTS IN NURSING HOMES	F5-1
09:15	Dreischulte, T. DATA DRIVEN QUALITY IMPROVEMENT IN PRIMARY CARE (DQIP): USING ROUTINE DATA TO IMPROVE THE QUALITY AND SAFETY OF PRESCRIBING IN PRIMARY CARE	F5-2
10:00	Pause	
10:20	Eickhoff, C. ARZNEIMITTELTHERAPIESICHERHEIT IN DER SELBSTMEDIKATION	F5-3(n.v.)
11:05	Schwalbe, O.; Braun, C.; Simons, S.; Jaehde, U. MORE THAN GOOD PRICES - PATIENT SAFETY IN DRUG THERAPY WITHIN A LARGE COLLABORATION OF COMMUNITY PHARMACIES	F5-4
11:50	Schrappe, M. ERKENNTNISINTERESSE UND INSTRUMENTE - WICHTIGE METHODISCHE FRAGESTELLUNGEN IN DER ARZNEIMITTELTHERAPIESICHERHEIT	F5-5(n.v.)

Fachsymposium Pharmakologie und Toxikologie

In Vitro Hautmodelle Als Alternative Pharmakologische Testsysteme

(SN 19.4)

Mittwoch**,** 06. Oktober, 2010

14:30	Begrüßung und Einführung Weindl, G.	
14:45	Hennies, H.C.; Torres, S.; Casper, R.; Weindl, G.; Ackermann, K.; Küchler, S.; Oji, V.; Traupe, H.; Schäfer-Korting, M.; Eckl, K.M. IN-VITRO MODELS FOR CONGENITAL KERATINIZATION DISORDERS	F6-1
15:20	Küchler, S.; Wolf, N.; Schäfer-Korting, M. IN VITRO WOUND HEALING MODELS	F6-2
15:55	Pause	
16:15	Merk, H. HAUTTUMORMODELLE	F6-3(n.v.)
16:50	Weindl, G. IN VITRO INFECTION MODELS OF LOCALIZED CANDIDA INFECTIONS	F6-4
17:25	Abschlussdiskussion	

Fachsymposium Pharmazeutische Biologie

Pflanzenextrakte im Spannungsfeld zwischen Rationaler Phytotherapie und Lebensmitteln bzw. Kosmetika

(SN 19.3)

Mittwoch**,** 06. Oktober, 2010

14:30	Begrüßung und Einführung Knöss, W.	
14:45	Steffen, C. KLINISCHE PRÜFUNGEN IM ABGRENZUNGSBEREICH	F7-1(n.v.)
15:20	Riedel, F. STOFFLISTE	F7-2(n.v.)
15:55	Pause	
16:15	Schraitle, R. VERKEHRSMÖGLICHKEITEN/VERTRIEBSOPTIONEN	F7-3(n.v.)
16:50	Stein, J. SEKUNDÄRE PFLANZENINHALTSSTOFFE ALS NUTRACEUTICALS – HYPE OR HOPE?	F7-4(n.v.)
17:25	Lohmüller, E.M. KOSMETIKA	F7-5(n.v.)
18:00	Abschlussdiskussion	

Fachsymposium Pharmazeutische/Medizinische Chemie

Mitgliederversammlung

(SN 19.4)

Mittwoch**,** 06. Oktober, 2010

13:15 Mitgliederversammlung

Fachsymposium Pharmazeutische Technologie

Dermale Therapie

(PK 4.7)

Mittwoch**,** 06. Oktober, 2010

14:15	Begrüßung und Einführung Müller-Goymann, C.C.	
14:25	Neubert, R.H.H. NEUE ERKENNTNISSE ZUR MOLEKULAREN UND MORPHOLOGISCHEN STRUKTUR DES STRATUM CORNEUM	F8-1
15:05	Schäfer, U.F. IN-VITRO METHODS TO DETERMINE THE DERMAL ABSORPTION. WHAT THEY CAN – WHERE ARE THEIR LIMITS?	F8-2
15:45	Lademann, J.; Richter, H.; Sterry, W.; Patzelt, A. PENETRATION AND STORAGE OF NANOPARTICLES IN THE SKIN	F8-3
16:25	Pause	
16:45	Daniels, R. DERMATOLOGICAL VEHICLES – CLASSICAL AND INNOVATIVE FORMULATION CONCEPTS	F8-4
17:25	Müller-Goymann, C.C.; Grüning, N.; van Hemelrijck, C. FORMULATION FOR THE TREATMENT OF SOLAR DAMAGES	F8-5
18:05	Abschlussdiskussion und Mitgliederversammlung der Fachgruppe Pharmazeutische Technologie	

Posterliste

Posterbeiträge

Pharmazeutische Technologie

Polymere

T001 Bauhuber, S.; Göpferich, A.; Breunig, M.
REDUCTIVELY DEGRADABLE LINEAR POLY(ETHYLENE GLYCOL)-POLY(ETHYLENE IMINE)-COPOLYMERS FOR THE DELIVERY OF NUCLEIC ACIDS

T002 Bertz, A.; Wöhl-Bruhn, S.; Bunjes, H.; Menzel, H.
DRUG DELIVERY SYSTEMS BASED ON MODIFIED HYDROXYETHYL STARCH

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PI-01

FROM SYSTEMS BIOLOGY TO PERSONALIZED MEDICINE – VISION, WISHFUL THINKING OR JUST A HYPE?

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New technologies to analyse genome wide DNA-sequence variations and the ability to carry out high-throughput measurements of RNA, protein expression or metabolite concentrations in blood and other tissues is changing biomedical research. The tremendous amount of data obtained during the course of disease progression or in response to drug treatments provide a basis for a mathematical description and the development of mathematical models of disease pathogenesis.

Instead of looking at individual components, we can now focus our attention on the interaction between the various components and the dynamics of biological systems. A network representation and analysis of the physiology and pathophysiology of biological systems and diseases is an effective way to study their complex behavior. Genetic, chemical or other environmental influences can trigger cascades of failures, which lead to the fragility and malfunctioning of cellular networks and to specific diseases.

Systems level approaches have a great potential to yield new insights into the molecular basis of drug action and to guide the improvement of drug safety and efficacy. The hopes and challenges associated with the application of systems biology and the development of a personalized medicine will be discussed.

PI-02

THE PRACTICE OF METABONOMICS/METABOLOMICS IN THE SEARCH FOR BIOMARKERS

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One of the aspirations of clinical innovation in the 21st century is contained in the concept of "personalised medicine" where the aim is to optimise the treatment regimen such that the right drug is given to the right patient at the correct dose. Central to the delivery of this concept is the assumption that patients can be profiled in some way, either *via* genetic data, or through phenotypes based on protein or metabolite signatures (proteomics/metabonomics) to derive specific biomarkes (or patterns of biomarkers). In the area of global metabolite profiling (metabonomics/metabolomics) this has resulted in the development of the idea of pharmacometabonomics where, by analogy to pharmacogenomics, predose metabolic phenotypes can be used to predict drug response.

The production of global metabolite profiles from biofluids and tissues as a means of studying the metabolic response of humans or other organisms to a toxic insult, or the development of disease, represents a major analytical challenge. Currently the bulk of the investigations in this field have used high field NMR spectroscopy or (increasingly) a separation technique combined with mass spectrometry (MS) (most often HPLC-MS and UPLC-MS, but also GC-MS, GCxGCMS and CE-MS). Validation and quality control in this type of work are essential, but by no means trivial, if useable data are to be obtained. Following profiling the data must then be interrogated using multivariate statistics so as to discover the potential biomarkers hiding in the forest of other metabolites, and then these must be identified and then investigated using sensitive, specific and fully validated, methods to confirm their utility.

The use of HP and UPLC-MS methods for biomarker discover and validation in metabonomic studies of disease models and in the investigation of nephro- and hepatotoxicity in rodents will be discussed in detail, and a road map for this type of study will be provided.

PI-03

AUSGETRÄUMT – INFEKTIONSKRANKHEITEN, IHR VERSCHWINDEN UND IHRE RÜCKKEHR IM 20. JAHRHUNDERT

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Zu den wichtigsten Entwicklungen in der europäischen Medizingeschichte des 20. Jahrhunderts gehört die Veränderung der Bedeutung von Infektionskrankheiten. Bis zum 1. Weltkrieg bildeten Krankheiten wie Tuberkulose, Cholera oder Typhus, verstanden als Volksseuchen, den Dreh- und Angelpunkt des öffentlichen Gesundheitswesens. Allerdings ging bereits seit dem Ende des 19. Jahrhunderts ihre epidemiologische Bedeutung zurück. Dieser Prozess hatte seinen Ursprung in einer allgemeinen Verbesserung der Lebensverhältnisse, welche durch medizinische Innovationen wie Serumtherapie, Schutzimpfungen und allmählich auch durch spezifische Therapien gefördert wurde. Mit der Verfügbarkeit von Sulfonamiden und fungalen Antibiotika kam insofern nach dem 2. Weltkrieg ein historischer Wandel zum Abschluss. An dessen Ende hatten chronische Erkrankungen des Herz-Kreislaufssystems, Diabetes und anderes mehr den Platz der klassischen Volksseuchen im öffentlichen Gesundheitswesen aber auch im Bewusstsein ihrer Zeitgenossen übernommen. In den 1980er Jahren verdichtete sich eine ganze Reihe von teilweise schon vorgängigen Veränderungen in ein neues Bedrohungsszenario, in dem Infektionskrankheiten nun wieder einen prominenten Platz einnahmen. Als wichtige Stichworte seien AIDS, Antibiotikaresistenzen, die Neudefinition chronischer Krankheiten als ansteckende und schließlich die Karriere von neuen ‚Volksseuchen' wie der Grippe genannt.

Wahrnehmung und Wirklichkeit sind jedoch zweierlei: Waren die Infektionskrankheiten jemals wirklich unter Kontrolle, inwiefern lassen sich die neuen und die alten Volksseuchen vergleichen, wie haben sich die Strategien ihrer Kontrolle im Laufe der Zeit verändert? Ausgehend von solchen und anderen Fragen möchte ich in meinem Vortrag dazu einladen, dass 20. Jahrhundert in seiner Gesamtheit als eine Epoche der Medizingeschichte zu begreifen.

PI-04

BACTERIAL INFECTIONS AT ATOMIC RESOLUTION

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Molecular mimicry is a common hallmark of the various strategies employed by pathogenic microorganisms during the infection of the human host. The skillful subversion of host cell processes by emulation can, for instance, allow adhesion and invasion into the host cell that are crucial steps during infection. We have been studying the mechanism of host cell invasion by the food-borne human pathogen *Listeria monocytogenes*, the causative agent of listeriosis. Using X-ray crystallography we have solved the structures of the so-called internalins, of which internalins A (InlA) and B (InlB) represent the two major listerial invasion proteins. While InlA directly interacts with human E-cadherin, promoting specific invasion into human epithelial cells, InlB recognizes and activates the receptor tyrosine kinase Met, the natural receptor for hepatocyte growth factor (HGF) allowing bacterial entry into a much wider spectrum of host cells. The structure of InlB in complex with human Met revealed InlB, which is structurally unrelated to HGF, perfectly mimics the function of the growth factor by activating its receptor by induced dimerization. To confirm the critical role of dimeric InlB in this process we used cross-linking experiments in which InlB-molecules were linked *via* disulfide bridges to form covalent InlB-dimers that showed enhanced Met activation surpassing that of HGF. We anticipate a potential pharmaceutical use of cross-linked InlB in wound-healing processes that rely on Met activation based cell scattering.
In a second example we present structural information on a new class of proteins from *Shigella sp.*, the cause of bacillary dysentery. These proteins act as perfect molecular mimics of guanosine nucleotide exchange factors involved in actin cytoskeleton dynamics, a central host cell process manipulated during bacterial invasion.

PI-05

MÖGLICHKEITEN UND GRENZEN INDIVIDUALISIERTER MEDIZIN

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Das Gesundheitssystem der Bundesrepublik Deutschland steht vor großen Herausforderungen. Die rasch alternde Bevölkerung mit höherer Krankheitsprävalenz, zunehmender Multimorbidität und immer komplexeren Behandlungsmöglichkeiten erfordert eine konsequente Qualitätsentwicklung und die Optimierung des Ressourceneinsatzes. Als eine Option wird international die Individualisierte Medizin (Synonym „Personalisierte Medizin“) diskutiert. *Mit ihr wird angestrebt, unter Zuhilfenahme modernster Diagnostik und durch Einsatz neuer, individuell auf die Bedürfnisse des Patienten ausgerichteter Therapieverfahren die Effektivität der Behandlung zu steigern, unerwünschte Effekte zu vermeiden, somit die Effizienz zu erhöhen und vermeidbare Kosten zu reduzieren.*
Insbesondere im Bereich einer Individualisierung der Therapie mit Arzneimitteln hat es in jüngster Zeit erhebliche Fortschritte gegeben. So konnte gezeigt werden, dass Patientinnen mit Brustkrebs, die einen genetischen Defekt in der CYP2D6 vermittelten Bioaktivierung von Tamoxifen haben, eine signifikant schlechtere Prognose aufweisen. Gleiches gilt für solche Patientinnen, die zusätzlich zu Tamoxifen mit Antidepressiva behandelt wurden, die ebenfalls dazu führen können, dass die Giftung von Tamoxifen nicht funktioniert.
Eine Umsetzung personalisierter Medizin in die tägliche Praxis erscheint deswegen machbar, weil die dafür notwendigen Techniken für einen breiten Einsatz verfügbar sind. Die Lagerung von biologischen Proben großer Patientenkollektive erfolgt in automatisierten Biobanken. Die analytischen Verfahren zur Diagnostik dieser Proben (Genetik oder Proteindiagnostik) erfolgt mit Hochdurchsatzmethoden. Analysen des humanen Genoms zeigen, dass neben Umweltfaktoren die genetische Variabilität über Veränderungen im Proteom und Metabolom entscheidend zum individuellen Erscheinungsbild multifaktorieller Erkrankungen beiträgt. Im Kontext der Etablierung der personalisierten Medizin verändern sich auch konzeptionelle und theoretische Grundlagen der Medizin. Es entstehen neue Fragen der Probanden-/Patientenethik.

PI-06

COLLOIDS AS VACCINE DELIVERY SYSTEMS - KOLLOIDE ALS IMPFSTOFFTRAEGER

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With current gene and protein technology it is now possible to identify specific regions of some whole organisms or cells which are likely to be recognized by the immune system, and to reproduce them synthetically as subunit vaccines. These so called epitopes are very safe because they are non-living but they also tend to be only poorly immune stimulating.

To improve the immunogenicity of a poorly immunogenic antigen, our approach is to use colloids as delivery systems. Liposomal delivery systems and related lipidic particles are thought to enhance the immune response by more closely mimicking a virus or microorganism due to the possibility of multimeric antigen presentation and their larger size compared to subunit antigens.

Our group has developed and characterised a range of colloidal delivery systems for the delivery of subunit vaccines:

- Mannosylated liposomes
- Adjuvant (Quil A) containing liposomes,
- Immune stimulating complexes (ISCOMs),
- Cationic ISCOMs (termed Pluscoms),
- ISCOM implants,
- Cubosomes.

In this presentation an overview will be presented about the various colloidal delivery systems our group has developed for the delivery of subunit vaccines. New results in this field, both on physico-chemical characterisation and immunological activity of these colloidal carriers, will be presented.

PI-07

THE GENOME AS A TOOL FOR CLINICAL PHARMACY

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The field of pharmacogenomics has seen some exciting advances in the recent past. The Human Genome Project and International HapMap projects have uncovered a wealth of information for researchers. This has lead to the discovery of clinically predictive germline genotypes (e.g. UGT1A1*28-irinotecan, TYMS TSER-fluoropyrimidines, CYP2D6-tamoxifen), germline haplotypes (e.g. VKORC1 Haplotype A-warfarin) and somatic mutations (e.g. epidermal growth factor receptor-gefitinib/erlotinib, KRas-cetuximab/panitumumab). The introduction of FDA approved pharmacogenetic tests and the initiation of genotype-guided clinical trials to treat cancer and heart disease have provided the first steps towards the integration of pharmacogenomics into clinical practice. It is also clear that there are many barriers to clinical application. These include expanding the science to understanding the pathways of genes that regulate a drug's activity. This information can be used as potential clinical biomarkers for selecting which drug or dosage is more likely to provide benefit to a patient. Genetic approaches are currently being used to assess the number of apparent genes/pathways involved in the cytotoxicity of a novel compound. Genomic tools can also be used to illuminate the mechanism(s) of action of compounds that behave in a pattern that is unique amongst existing drugs. There are also critical non-science issues, such as integration of new tests into health systems, changing old habits to allow application of new data, and the reality that the cost of both testing and the therapeutic options are a key driver in health care. As the scientific evidence matures, we must think beyond our favorite aspect of translational science if we are to overcome the many obstacles to delivering more careful selection of cancer therapy.

PI-08

DIE BEDEUTUNG VON STAMMZELLEN FÜR DIE DIABETES-THERAPIE

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Für die Stammzelltherapie des Diabetes mellitus und anderer Erkrankungen stehen prinzipiell verschiedene Stammzell-Reservoirs mit jeweils spezifischen Vor- und Nachteilen zur Verfügung. **Humane embryonale Stammzellen** sind einfach zu isolieren und zu vermehren und können in insulinproduzierende Zellen differenzieren. Im Hinblick auf die ethische Problematik bei der Gewinnung von Stammzellen aus menschlichen Embryonen brachte der Nachweis, dass adulte somatische Körperzellen durch gezielte Manipulation in pluripotente Zellen mit embryonalen Charakter zurückgeführt werden können, einen entscheidenden Durchbruch, sogenannte **induzierte pluripotente Stammzellen** (IPS). Nach wie vor steht dem klinischen Einsatz aber das erhebliche tumorigene Potenzial dieser Zellen entgegen. Die gezielte Differenzierung **adulter mesenchymaler Knochenmarksstammzellen** ist technisch anspruchsvoller als die embryonaler Stammzellen, dafür können sie einfach und ethisch unproblematisch gewonnen werden. Erst seit kurzem weiß man, dass in den meisten Geweben kleine Menge **adulter gewebsspezifischer Stammzellen** vorhanden sind, deren Identifizierung und Isolierung sich aber schwierig gestaltet. Aus dem Pankreas wurden bislang duktale Stammzellen (aus dem Epithel der Pankreasgänge) und insuläre Stammzellen gewonnen, und es wurde gezeigt, dass aus humanen duktalen Stammzellen insulinproduzierende Zellen differenzieren können. Diese können aber derzeit in noch nicht ausreichender Menge für den klinischen Einsatz *in vitro* hergestellt werden. Ein interessanter Aspekt ergibt sich aus der Beobachtung, dass das GLP-1 als Wachstumsfaktor für duktale Vorläuferzellen und für Betazellen fungiert. Damit können humanes GLP-1 und dessen Analoga zur Expansion von pankreasspezifischen adulten Stammzellen genutzt werden. Zumindest im Tierversuch konnte mit Exenatide auch schon eine Zunahme der Betazellmasse und eine Verbesserung der Diabeteseinstellung erzielt werden.
Wir sind zusammenfassend aktuell an einem Punkt angekommen, an dem man mit Bestimmtheit davon ausgehen kann, dass in der Zukunft Strategien entwickelt werden können, um voll funktionelle insulinproduzierende Betazellen, aus welcher Quelle auch immer, für den therapeutischen Einsatz bei Patienten mit Diabetes mellitus zu generieren.

PI-09

PHARMACOLOGICAL INHIBITORS OF CYCLIN-DEPENDENT PROTEIN KINASES RELEVANT TO CANCER.

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Phosphorylation of serine, threonine and tyrosine residues represents one of the most common post-translational mechanisms used by cells to regulate their enzymatic and structural proteins. Alterations in the phosphorylation of proteins represent a frequent feature associated with human disease. This is the reason for an exponentially growing investment in the discovery, optimization and therapeutic evaluation of small molecular weight, pharmacological inhibitors of protein kinases. It is estimated that 30-35% of drug discovery programs in the pharmaceutical industry currently target a protein kinase! Presently, over 130 kinase inhibitors are undergoing clinical evaluation against diseases such as cancers, inflammation, diabetes, and neurodegeneration.
Among the 518 human kinases, our laboratory has focused its efforts on several families of serine/threonine kinases: *cyclin-dependent kinases* (CDKs), *glycogen synthase kinase -3* (GSK-3 and its *Plasmodium* ortholog PfGSK-3), *casein kinases 1* (CK1) and *dual-specificity tyrosine phosphorylation regulated kinases* (DYRKs). In particular, CDKs have attracted considerable interest because of their numerous key physiological functions such as regulation of cell division cycle, apoptosis, multiple neuronal activities, pain signaling, insulin release, transcription, RNA splicing, etc... Their involvement in human diseases such as cancers & leukemias, chronic & acute neurodegenerative disease (Alzheimer's and Parkinson's diseases, stroke), kidney diseases (glomerulonephritis, polycystic kidney disease), inflammation, type 2 diabetes, viral infections, unicellular parasites has been widely investigated and will be summarized.
To illustrate the potential of pharmacological inhibitors of these kinases, we will describe a selection of CDK inhibitors derived from the clinical phase 2 drug roscovitine. The selectivity and intracellular mechanism of action of these compounds, their chemical synthesis and their pharmacological properties have been extensively studied and will be presented as representative examples of the multiple effects of kinase inhibitors in cells, tissues and organisms, and their therapeutic potential against selected cancers. The key role of the survival factor Mcl-1 and the transcription factor Myc in the action of CDK inhibitors will be highlighted.

PI-10

HYPERFORIN – FROM THE HERB TO THE MOLECULE AND TARGET

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More than ten years ago we got interested in the antidepressant mechanism of Hypericum extract. We soon found out that similar to other antidepressants hypericum extract inhibits the neuronal uptake of serotonin, norepinephrine, and dopamine and that hyperforin is the responsible constituent. However, hyperforin was not an inhibitor of the respective transporters but inhibited neuronal uptake by elevating free intracellular Na^+, thereby decreasing the sodium gradient over the neuronal membrane, the driving force for the amine transporter. In a next step we identified TRPC6 channels as the specific targets of hyperforin not only mediating Na^+ but also Ca^{2+} influx. By breaking down the pharmacophor of hyperforin to simple substituted phloroglycinol derivatives we could demonstrate that the compounds share its target at the TRPC6 channel probably with its physiological activator diacylglycerol. Since antidepressant activity has recently been linked with neuroplasticity and TRPC6 channels are relevant for neuroplasticity phenomena our findings of substantial effects of hyperforin on neuritic outgrowth would suggest that it works as antidepressant not only by enhancing the extracellular levels of monoamines but also by directly activating neuronal plasticity. Again, these effects are only seen for hyperforin but not for other constituents of hypericum extract. TRPC6 channels not only promote neuronal differentiation but also reduce keratinocyte proliferation and promote their final differentiation. Our findings that hyperforin stimulates keratinocyte differentiation by activating TRPC6 channels are finally suggesting that its use in dermatological diseases e.g. atopic dermatitis might also be explained by activation of the same target as for its use as an antidepressant drug.

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PI-11

ENTWICKLUNG NEUER ANTITUMORALER METALLKOMPLEXE

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Die chemische Diversität der Metallverbindungen eröffnet eine Fülle an Möglichkeiten für die Entwicklung antitumoraler Wirkstoffe. Derzeit werden u. a. Verbindungen von Ruthenium, Gallium und Lanthan in präklinischen und teilweise bereits in klinischen Studien untersucht. Vor allem die Rutheniumverbindung NKP1339 hat bereits bei terminalen Tumorpatienten therapeutische Aktivität gezeigt. Die Wirkmechanismen sind vermutlich ebenso unterschiedlich wie das chemische Verhalten der Metallelemente (v. a. hinsichtlich Koordination- und Redoxchemie), die zytotoxischen Potenzen dieser Verbindungen korrelieren jedoch nicht notwendigerweise mit der Aktivität in vivo.

Im Falle der heterozyklischen Rutheniumkomplexe wurden lange Zeit vor allem Interaktionen mit Serumproteinen und DNA untersucht, doch blieb v. a. die Relevanz der letzteren fraglich. Derzeit wird an der Identifizierung anderer, möglicherweise ausschlaggebender Targets gearbeitet. Weiters wurde die Aktivität im Tiermodell erhärtet, und die klinischen Studien wurden wieder aufgenommen.

Der oral bioverfügbare Galliumkomplex KP46 erwies sich als besonders wirksam in der Primärzellkultur des malignen Melanoms, und eine erste klinische Studie erbrachte Hinweise auf eine Aktivität im Nierenzellkarzinom. Die lipophile Ligandensphäre dieser Verbindung hat nicht nur Auswirkungen auf die Biodistribution, sondern möglicherweise auch auf den Wirkmechanismus, der von anderen Galliumverbindungen abzuweichen scheint.

Das antitumorale Potential der Lanthaniden wurde bis vor kurzem kaum untersucht. Die Lanthanverbindung KP772 wurde jedoch in vivo als wirksam erkannt, und multidrug-resistente Zellmodelle erwiesen sich als hypersensitiv gegenüber dieser Verbindung. Durch Langzeit-Behandlung lässt sich ein Verlust des MRP-1 und eine Resensitivierung gegenüber organischen Chemotherapeutika erreichen, was völlig neue Perspektiven für die Resistenz-Überwindung eröffnet.

Auch auf dem Gebiet der Platinverbindungen können neue Fortschritte berichtet werden. So ergaben zuvor nicht untersuchte Struktur-Wirkungs-Beziehungen rund um die Oxaliplatin-Struktur Aufschlüsse für die Entwicklung wirksamer Derivate, während pH-sensitive Platin-Komplexe sowie Platin–Oxim-Komplexe mit aktiven trans-Isomeren als weitere vielversprechende Innovationen untersucht werden.

PI-12

STIMULATORS AND ACTIVATORS OF SOLUBLE GUANYLATE CYCLASE: FROM BENCH TO BEDSIDE

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The soluble guanylate cyclase (sGC) is a key signal-transduction enzyme in the cardiovascular system and activated by NO. It became apparent that many cardiovascular diseases are associated with a dysfunction of the NO/sGC system. Importantly, two different forms of sGC exist in vivo, the native and heme-free sGC. sGC activators, such as cinaciguat (BAY 58-2667) are capable of selectively activating the haem-free enzyme via binding to the enzyme's haem pocket. These new compounds selectively target the dysfunctional sGC that is prevalent under disease conditions. Cinaciguat has demonstrated efficacy in patients with acute decompensated heart failure (ADHF), reducing pre- and afterload and increasing cardiac output. A clinical IIb study for the indication of ADHF is currently underway. sGC stimulators, such as BAY 41-2272 and riociguat (BAY 63-2521), show a dual mode of action: they sensitize sGC to the body's own NO while also directly stimulating sGC independently of NO. They may be beneficial in the treatment of a range of cardiovascular and non-cardiovascular disorders. Riociguat had beneficial effects on pulmonary haemodynamics, right heart hypertrophy, and remodeling of the pulmonary vasculature in animal models of pulmonary hypertension (PH). Riociguat has demonstrated efficacy in patients with PH, reducing pulmonary vascular resistance and increasing cardiac output. A clinical phase II study shows that riociguat significantly improves exercise capacity, hemodynamic and symptoms in patients with pulmonary arterial hypertension and, remarkably, also in patients with chronic thromboembolic pulmonary hypertension. Based upon the promising results, global phase III trials of riociguat in patients with different forms of PH are underway.

PI-13

POLYMERS FOR THE CONTROL OF CELL MATERIAL INTERACTIONS ON THE MICRO- AND NANOSCALE

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Numerous biomedical applications depend critically on the controlled interaction of cells with materials. Cellular responses upon material contact are in tissue engineering and regenerative medicine applications of utmost significance since they decide over cell proliferation and differentiation. A strategy towards the control of these processes that was developed rather successfully in recent years is a biomimetic approach that relies on mimicking natural interaction mechanisms. For such applications, polymers have been developed that mimic macromolecular components of the extracellular environment[1]. They bind to cell surface receptors that allow for cell signalling thus giving us the opportunity to control basic cellular processes. In these cases we provide cells with a microscopic environment that is larger than the cellular dimensions. Polymers play a similarly prominent role for the development of nanoscopic drug delivery carriers the dimensions of which are substantially smaller than that of a cell. Especially the search for novel materials that allow for specific cell entry has been intensified recently[2]. Such materials need to host substances like nucleic acids and have concomitantly to allow for specific interactions with cells.
The requirements that applications on the micro- as well as on the nanoscale pose have tremendous implications for a polymer. While we have mastered to implement most basic requirements, like the ability to degrade, we are still trying to design polymers with a maximum degree of functionality. This is not a trivial task since we need to outfit them with almost mutually exclusive properties. On the one hand the polymers need to possess a certain degree of camouflage to avoid intrinsic unspecific interactions with cells especially off-target cells. Yet on the other hand they must be visible to their target with a maximum degree of selectivity and affinity. This apparent paradox can only be resolved when we implement spatial and temporal variability into the structure of polymers. Along these design criteria a number of materials have been developed in recent years for sophisticated applications that will be reviewed during the presentation.

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[2] W. Hild et al., PNAS, 107 (2010) 10667-10672.

PI-14

EVIDENCE-BASED COMPLEMENTARY MEDICINE – A CONTRADICTION IN TERMS?

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Complementary medicine is popular. This is particularly true of Germany where about two thirds of population use such treatments each year. This level of popularity means that we should have good evidence about the effectiveness and risks of complementary medicine, and that we need to act according to this evidence. Summarizing the current knowledge, we observe the following:

- Only few therapies are demonstrably effective.
- Some seem to be demonstrably ineffective.
- For many treatments, the effectiveness is unknown.
- The risks are frequently under-researched.

It follows that, in this area, more research is urgently needed. Evidence-based complementary medicine must not remain a contradiction in terms. As in all of medicine, only those methods should be employed that demonstrably generate more good than harm.

Keynotes

Key1-1

MICRO SYSTEMS FOR FORMULATION- AND PROCESS-PARAMETER-SCREENING

Kwade, A.[1], Büttgenbach, S.[2], Klages, C.-P.[3], Krull, R.[4], Franco-Lara, E.[4], Kampen, I.[1], Müller-Goymann, C.[5], Bunjes, H.[5], Radespiel, R.[6], Kähler, C.[7], Augustin, W.[8], Scholl, S.[8]
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Micro systems feature many advantages like very effective mass and heat transfer, fast mixing, narrow residence time distributions, small educt and system volumes, very small product losses as well as small cleaning expenditure. The DFG research group „Micro Systems for particular Life-Science-Products“ develops and investigates micro systems for two different screening applications: On one side a micro system for the processing and formulation of nano scaled drug delivery systems (lipid nano particles) including their loading and on the other side micro bio reactor systems for the determination of advantageous conditions for cells cultivation. Key aspects of the research are the development and design of geometries and surfaces of micro systems for these two applications, the specific adjustment of stresses, mass and heat transport (e.g. oxygen and substrate delivery), the minimization of wear and adhered particles as well as securing Cleaning in Place (CIP). Moreover, experimental and numerical methods are developed with which the micro systems can be characterised and evaluated regarding fluid flow, particle stressing, wear, fouling and cleaning.
The capability of the micro systems will be presented by discussing results of dispersing nano particles and emulsification of lipids in different high pressure micro channels as well as of the cultivation of yeast cells in micro bio reactors. The different high pressure geometries show different properties and advantages depending on the kind of process, dispersing or emulsification. Beside the process investigations the fluid flow in the different micro systems was investigated experimentally by Micro-Particle Image Velocimetry (μPIV) and numerically by CFD-simulations. Moreover, the micro systems were characterised regarding wear applying different surface coatings as well as adhesion of particles and micro organisms.

Key1-2

MICROBIOREACTORS – A SCREENING-TOOL FOR BIOLOGICAL PROCESSES

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In the last years the well-established usage of conventional microtiterplates is fundamentally refined by the introduction of micro-scale bioreactors as suitable tool for a wide range of interesting applications. A diffusion based microbioreactor (MBR) system operated with a reaction volume of 8 μL is developed and characterized to intensify the process understanding in microscale cultivations. The device is composed of a glass bottom and a microstructured top layer made of gas permeable poly(dimethylsiloxane) (PDMS) using UV-depth and soft lithography. The potential of the MBR as screening tool for biological processes is evaluated. The advantage of the designed MBR is the use for the continuous cultivation mode by integrating online measurement technique for dissolved oxygen (DO) and optical density (OD) to measure biomass growth. The bioreactor geometry was chosen to achieve homogeneous flow during continuous process operation. CFD simulation data used for geometry design were verified via micro-particle-image velocimetry (μPIV). In the used MBR geometry no concentration gradients occurred along the entire reaction volume because of rapid diffusive mixing. A homogeneous medium flow inside the growth chamber of the MBR can be realized. Undesirable bubble formation before and during operation was reduced by using degassed medium as well as moistened and moderate incident air flow above the PDMS-membrane. Due to this a passive oxygen supply of the culture medium in the device is ensured by diffusion through the PDMS-membrane. The oxygen supply itself was monitored online via integrated DO sensors based on a fluorescent dye complex. An adequate overall volumetric oxygen transfer coefficient as well as the mechanical stability of the device has been accomplished for a membrane thickness of 300 μm. Experimental investigations consider measurements of DO and OD (online) for the biomass growth and the concentration of glucose and ethanol (offline) via HPLC in a modified Verduyn growth medium. The used model organism *Saccharomyces cerevisiae* DSM 2155 tended to strong reactor wall growth. The reaction kinetics of the model organism estimated in the MBR were compared with data of a conventional 1L-stirred tank bioreactor system.

Key1-3

PREPARATION OF LIPID NANOPARTICLES IN MICRO-STRUCTURED SYSTEMS

Bunjes, H.[1], Fehr, S.[1], Finke, J. H.[1], Schur, J.[1], Müller-Goymann, C. C.[1], Lesche, C.[2], Büttgenbach, S.[2], Gothsch, T.[3], Kwade, A.[3], Jasch, K.[4], Huzhalska, V.[4], Kulik, A.[4], Augustin, W.[4], Scholl, S.[4]
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Lipid nanoparticles are promising carrier systems for the delivery of lipophilic drugs. Their potential benefits in delivering insoluble substances such as sun protection pigments or water soluble biomacromolecules like proteins are also being explored. The most common way of preparing such particles is by high-pressure homogenization of a coarse, premixed emulsion using the melted matrix materials if solid lipids are to be processed. This manufacturing method can easily be scaled up but the preparation of very small quantities, as would be beneficial for formulation screening, is currently not established. It was thus our aim to explore the use of microsystems for the small-scale preparation of lipid nanoparticles with at least similar product quality as high-pressure homogenized dispersions. Different types of microsystems were used instead of conventional homogenization valves to disperse the particles into the colloidal state. One approach employed different types of customized silicon microchannels in which the lipid phase is dispersed by different types of flow. The emulsification efficiency highly depended on the geometry of the channels as well as on the pressure applied. With multiple passes, mean particle sizes in the range of 100-200 nm could be prepared. Alternatively, the lipid phase was dispersed by extrusion of the coarse premix through nanoporous filters. Also with this method, particles with a mean size around 120 nm could be obtained depending on the pore size, extrusion conditions and the composition used. In optimized cases, extrusion resulted in very narrow particle size distributions after multiple passes. Very small batch sizes (≤1 ml) could be prepared when a handheld extruder was used but this method was more sensitive to sample composition than larger-scale extrusion. For the crystallization step, that is required for the preparation of solid lipid nanoparticles, the use of a micro heat exchanger, allowing very high cooling rates, was established. While the continuous crystallization of different triglyceride-based compositions was easily possible, a wax-based dispersion led to plugging of the heat exchanger channels during the crystallization process.

Key2-1

CHEMICAL RNOMICS - THE SEARCH FOR NEW REGULATORY RNAS

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Riboswitches are RNA sequences that directly sense – without the help of proteins – metabolite concentrations and thereby induce changes in gene expression. To date, there exists no biochemical screening method for isolating naturally occurring unknown riboswitches from biological samples.
We are establishing a direct and unbiased experimental approach for the isolation of unknown riboswitches. This approach combines methods developed in functional proteomics and experimental RNomics and will allow isolating functional RNAs solely by their ability to bind a metabolite, without any prior knowledge about primary sequence or higher-order structure. The basis of this strategy is photoaffinity tagging. Metabolites are, by chemical derivatization, converted into photoaffinity probes that contain a photoreactive group and an affinity tag (e.g., biotin). After binding to their target RNAs, the probes will be photocrosslinked by UV irradiation, thereby creating a covalent linkage between RNA and affinity tag. After affinity chromatography, the isolated RNAs are amplified by a combination of adapter ligations and tailing methods. Adapter sequences are designed for direct use in next generation sequencing. Isolated metabolite-binding RNAs will be characterized biochemically.

Key2-2

FLUORESCENCE SPECTROSCOPY BASED ANALYSIS OF SMALL INTERFERING RNA INTEGRITY DURING FORMULATION, TRANSFECION, AND INTRACELLULAR DISTRIBUTION

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The field of therapeutic oligonucleotides has rebounded due to the development of small interfering RNAs (siRNAs). An upsurge is taking place in industry and academia alike, owing to the fact that applications of siRNA in life science research are of outstanding importance in their own right. A key feature of siRNAs is the reprogramming of a complex cellular mechanism, originally evolved to control gene expression. Reprogramming by siRNA includes redirecting catalytic RNase activity to a target mRNA of choice. Despite impressive advances in pharmaceutical chemistry, biology, and technology, the efficiency of different siRNAs, once delivered into the cell, is still variable. Moreover, most of the delivered siRNA material does not reach its final destination in the catalytic complex intact. Conjugation of siRNA to dyes that form donor-acceptor pairs in fluorescence resonance energy transfer (FRET), allows tracing the distribution of intact siRNA during nanoscale formulation and across the various subcellular compartments. Intact siRNAs performs a movement into the nucleus and out again, before being subject to degradation, which leads to perinuclear accumulation of siRNA debris. We are currently characterizing differential distribution patterns of siRNAs from different formulations, with various chemical modifications, and of distinct biological activity.

Key2-3

REPRESSION OF THE PROTO-ONCOGENE PIM-1 BY MIR-33A

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The constitutively active serine/threonine kinase Pim-1 is upregulated in several cancer types, mainly based on the action of several interleukines and growth factors at the transcriptional level. In contrast, a regulation of Pim-1 by miRNAs, which may well add to the differential expression in tumor versus normal cells, has not been reported so far. Tumor relevant miRNAs may either act as oncogenic miRNAs or exert tumor suppressor activity. Here we newly establish miR-33a as a miRNA with tumor suppressor activity, and demonstrate that it acts through inhibition of the oncogenic kinase Pim-1 as a natural miR-33a target. A screen for miRNA expression in K562 lymphoma and LS174T colon carcinoma cells revealed only low endogenous miR-33a levels relative to other miRNAs, such as the oncogenic miR-17-5p or miR-20a. Transfection of the two cell lines with miR-33a mimics reduced Pim-1 levels. In contrast, another kinase involved in cell cycle regulation and predicted by TargetScan 5.1 with an even higher score, Cdk6, was found to be no target for miR-33a regulation. Seed mutagenesis of the Pim-1 3'-UTR in a luciferase reporter construct demonstrated the specificity of the miR-33a-dependent downregulation. The transfection of K562 and LS174T cells with a miR-33a mimic decelerated proliferation without inductin of apoptosis. The persistence of this effect was comparable to that of an siRNA-mediated knockdown of Pim-1. We further provide evidence for a role of Pim-1 in promoting cell cycle progression at G1- to S-phase and G2- to M-phase transitions. In conclusion, we demonstrate the potential of miR-33a to act as a tumor suppressor miRNA and identify an underlying mechanism based on deceleration of cell cycle progression upon downregulation of Pim-1, which suggests miR-33a replacement therapy through forced expression as a novel therapeutic strategy.

Key3-1

PARADOCKS - A FRAMEWORK FOR MOLECULAR DOCKING WITH POPULATION-BASED METAHEURISTICS

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Molecular docking is a simulation technique that aims to predict the binding pose between a ligand and a receptor. The resulting multi-dimensional continuous optimization problem is practically unsolvable in an exact way. One possible approach is the combination of an optimization algorithm and an objective function that describes the interaction. The software is designed to hold different optimization algorithms and objective functions. At the current stage, an adapted particle-swarm optimizer (PSO) is implemented. Available objective functions are: (i) the empirical objective function p-Score, and (ii) an adapted version of the knowledge-based potential PMF04. We tested the docking accuracy in terms of reproducing known crystal structures from the PDBbind core set. For 73 % of the test instances the native binding mode was found with an rmsd below 2 Å. The virtual screening efficiency was tested with a subset of 13 targets and the respective ligands and decoys from the directory of useful decoys (DUD). ParaDockS with PMF04 shows a superior early enrichment. The here presented approach can be employed for molecular docking experiments and virtual screenings of large compound libraries in academia as well as in industrial research and development.

Key3-2

SEEING THE WOOD, NOT ONLY THE TREES - SYSTEMS CHEMICAL BIOLOGY

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The vast majority of virtual screening approaches aims to identify compounds that are active against a particular target. Such a reductionist approach usually does not consider both the interaction of targets within biological systems and possible polypharmacological activities of compounds.
This talk will give a general overview of these concepts and recent successes in applying them.

Key3-3

PHARMACOPHORE-BASED VIRTUAL SCREENING: AN EFFICIENT TOOL FOR BIO-ACTIVITY PROFILING AND AFFINITY PREDICTION

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Virtual screening using three-dimensional arrangements of chemical features (3D pharmacophores) has become an important method in computer-aided drug design. Although frequently used, considerable differences exist in the interpretation of these chemical features and their corresponding 3D overlay algorithms. We have recently developed an efficient and accurate 3D alignment algorithm based on a pattern recognition technique [1]. In the presented work, we extend this algorithm to be used for high-performance virtual database screening and investigate, whether applying this geometrically more accurate 3D alignment algorithm improves virtual screening results over conventional incremental n-point distance matching approaches. Additionally, the application of pharmacophore-based virtual screening algorithms for drug-repurposing and activity profiling will be discussed.

[1] G. Wolber, A. Dornhofer, and T. Langer. Efficient overlay of small molecules using 3-D pharmacophores. J. Comput.-Aided Mol. Design, 20(12): 773-788 (2006)

Key4-1

BIOENGINEERING OF GLUCORAPHANIN FROM BROCCOLI

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Epidemiological studies have demonstrated reduced risk of developing cancer upon consumption of diets rich in cruciferous vegetables. Key players in this chemoprevention are the natural products glucosinolates, in particular the methionine-derived glucoraphanin which is highly abundant in broccoli. Improved nutrition by functional foods or health-promoting dietary supplements is an attractive means for prevention of lifestyle-based diseases. Towards this goal, we have transferred the entire glucoraphanin biosynthetic pathway consisting of thirteen genes from Arabidopsis into the non-cruciferous tobacco by transient expression. The engineering involves the chloroplast-localized chain elongation machinery (5 genes) that converts methionine to dihomomethionine, and the cytosolic, ER-anchored core structure pathway ((8 genes) that converts dihomomethionine to the glucoraphanin. Transport engineering is important to ensure efficient channeling of intermediates between compartments and proper storage of end product to prevent feedback inhibition, but not much attention has so far been given to this aspect of engineering. Our progress in development of a technology platform for transport engineering will be discussed, as will our technology platform for engineering plant pathways into yeast.

1) Mikkelsen et al. (2010) Reconstitution of the glucoraphanin biosynthetic pathway. Molecular Plant (in press)

Key4-2

ENGINEERING *ARTEMISIA ANNUA* FOR ARTEMISININ PRODUCTION

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Artemisinin (also called qinghaosu) is a sesquiterpene lactone with an unusual 1,2,4-trioxane ring structure. It was isolated from the herb *Artemisia annua* L. by Chinese scientists in an effort of searching for novel antimalarial drugs in the 1970s. Artemisinin derivatives have provided the basis for the most effective treatments of multi-drug resistant and cerebral malaria, particularly in the form of artemisinin-based combination therapies (ACTs) which are advocated by the WHO in order to reduce the odds of resistance development. Artemisinin derivatives inactivate or kill gametocytes of *Plasmodium spp.* by inhibition of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) after activation by iron ions. Besides the antimalarial activity, artemisinins have been reported to possess antiviral, anticancer and antischistosomal activities.

A. annua is the only plant species known to synthesize and accumulate artemisinin. Since the detection of antimalarial activity of artemisinin, a lot of efforts including chemical synthesis, plant cell cultures, hairy roots and fermentation of engineered microorganism etc. have been made to increase the production of this compound. However, none of them are competitive and the *A. annua* plant is still the only commercial source of artemisinin. The low content of artemisinin in *A. annua*, ranging from 0.1to 1% of dry weight, makes artemisinin relatively expensive. In addition, it is hard to meet the demand of over 100 million courses of ACTs per year. Therefore, one of the most promising approaches to reduce the price of ACTs is metabolic engineering of the *A. annua* plant to obtain higher artemisinin content in the transgenic lines. In the past decade, we have established an *Agrobacterium*-mediated transformation system of *A. annua* and successfully transferred a number of genes related to artemisinin biosynthesis into the plant. Various aspects of these efforts will be presented.

Key4-3

LONG-TERM STORAGE OF UNDIFFERENTIATED PLANT CELLS FOR PRODUCTION OF HIGH VALUE SUBSTANCES

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Medicinal plants have been always considered a healthy source of life and today numerous pharmaceutically active substances are at least based on chemical structures which have been isolated from plants. Due to the huge chemical complexity of the substances of interest in many cases the biosynthetic pathway requires subsequent chemical modifications which are catalysed by specific enzymes. Therefore the specific plant cells which comprise these sets of enzymes are the best resources for the supply of the natural substance as seen for paclitaxel - a secondary metabolite isolated from *Taxus sp.*.
Moreover it has been shown that the production of secondary metabolites with plant cell fermentation – e.g. the production of paclitaxel - not only is valuable but at the same time allows the supply of the substance of interest at high quality.

For a robust and reliable industrial scale production process consistency and stability of the used cell material is required. In case of undifferentiated plant cells it has been shown that long-term storage of the material can be performed by cryopreservation. This well known technology is a method to conserve living cells but it allows to secure cells at a defined status and to recover this status even many years later, too. As a consequence every new production cycle can be started from plant material which has been recovered from cryopreserved cells thus allowing for a high batch-to-batch consistency. This procedure has been applied successfully for the paclitaxel production since 1997 and is still ongoing.

Although long-term storage of plant material is feasible for many plant species – our cell bank comprises cryopreserved material from hundreds of different plant species - the substance profiles prior and post freezing have to be investigated seriously case by case.
Stability tests performed at Phyton indicate that cryopreserved cell banks are a valuable source for the production of natural-based actives to be used in the pharmaceutical and health care industry.

Key5-1

INDIVIDUALIZATION OF ANTICANCER DRUG DOSING BASED ON PHARMACOKINETIC PRINCIPLES

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It is generally believed that for anticancer drugs the maximal tolerable dose is also the most effective dose, although this postulate is not necessarily true for all drugs. In oncology, many drugs are administered in doses very close to the maximum tolerated dose and, therefore, display a narrow therapeutic range.
In clinical practise, dosing of cytostatic drugs is based on body surface area (BSA). The rationale for this procedure is that the metabolic rate of the body as well as the volume of the extracellular space correlates well with BSA. Thus, as metabolic rate often correlates with the drugs` clearance and extracellular volume correlates with the volume of distribution of not too lipophilic drugs, BSA can be used to achieve comparable AUC`s as well as maximum concentrations (C_{max}) for most of the patients. Although this practise has been questioned in general, in heterogeneous populations BSA-dosing is the method of choice today as long as other approaches are not tested and validated. As an alternative, allometric scaling was suggested for children as a general approach of size-adjusted dosing.
For drugs being excreted mainly by glomerular filtration, formulae for dosing based on glomerular filtration rate were developed and evaluated. For carboplatin, the method of defining a target AUC and calculating the dose based on renal function is now established in clinical routine.
However, for many situations drug monitoring is recommended. For example, it is mandatory to adjust the administration of the leucovorin rescue after high-dose methotrexate (Mtx) therapy according to Mtx plasma concentration measurements. In leukaemia patients, asparaginase activity has to be controlled due to possible formation of inactivating antibodies. Dose individualisation of Busulfan based on plasma concentration measurements is done in some clinical centres. For patients receiving high-dose therapy before bone marrow transplantation, it would be desirable to have plasma concentrations of the cytostatic drugs in order to improve safety of these very risky treatment regimens. However, it is an analytical and logistic challenge to monitor drugs like cyclophosphamide and their highly unstable active metabolites in clinical routine.

Key5-2

INDIVIDUALIZATION OF ANTICANCER DRUG TREATEMENT BASED ON PHARMACOGENETIC FACTORS

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Genetic alterations are pivotal events in the development of neoplastic diseases. With the advent of extremely sensitive and broad high throughput gene analysing techniques such as fine tiling comparative genomic hybridization (CGH) and deep sequencing methods the tumor genome is now readily accessible. The identification of genetic aberrations and the characterization of their functional consequences have created marked improvements for cancer diagnostics, treatment stratification, and have even resulted in new and specifically targeted therapies. One of the great milestones in this respect was the development of the Bcr-abl-kinase inhibitor imatinib (Gleevec®) which is now an irreplaceable drug in the treatment of several types of Philadelphia chromosome-positive leukemia as well as subsets of gastrointestinal stromal tumors. On the other hand genetic alterations also affect genes that are involved in the metabolism and excretion of anticancer drugs. For instance, nucleoside analogs such as mercaptopurine or thioguanine which are mainstays for treatment of acute leukemias are heavily metabolized to active and inactive compounds. Genes involved in these processes include, most notably, thiopurine S-methyltransferase (TPMT), but other genes, i.e the drug transporter ABCC4 seem to be involved in thiopurine disposition as well. Genetic variation in these genes results in either toxicity or treatment failure. Taking all this into account, successful cancer treatment relies on a good knowledge of the genetic driving forces of progression of the individual tumor as well as the genetic variability of genes that affect disposition of the individually selected drugs at the tumor site. Considering the need for individual tumor testing, only few genetic diagnostic tools are available for routine use and mandatory or recommended by drug regulation authorities. And even those do not entirely reflect the genotype-phenotype correlation as has recently been shown for the TPMT testing, which denotes the complexity of these processes. The possibility of whole tumor genome sequencing will certainly propel individualization of tumor treatment, but for a whole understanding genetic testing ultimately needs to be combined with miRNA pattern analysis, tumor epigenetics and the identification of treatment guiding biomarkers.

Key5-3

INDIVIDUALIZATION OF ANTICANCER DRUG THERAPY USING BIOMARKERS

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Pharmacokinetic/pharmacodynamic principles are increasingly applied in order to to define and optimise dosage regimens. In oncology, this methodology has not been used widely due to the lack of pharmacodynamic markers that can be frequently measured. In contrast to the classical cytotoxic drugs, for most targeted drugs biomarkers are available of which some are easily measurable in the patients´ plasma.
This approach seems to be particularly promising for drugs with antiangiogenic properties which have been shown to influence blood pressure and plasma concentrations of various proteins (VEGF-A, soluble VEGFR-2 and VEGFR-3) The response to these biomarkers has been associated with clinical outcome, particularly in renal cell carcinoma patients. Therefore, it seems promising to integrate biomarker concentrations as pharmacodynamic markers into PK/PD models.
We conducted an explorative study in healthy subjects to investigate the concentration-effect relationship for sunitinib and its active metabolite SU12662. Based on drug and biomarker measurements, we developed semi-mechanistic pharmacokinetic/pharmacodynamic (PK/PD) models using NONMEM (Lindauer et al. 2010). Recently we have shown that these models can also be used to predict drug and metabolite concentrations as well as biomarker response in patients with metastatic colorectal cancer patients (see Kanefendt et al., this conference). The drug, metabolite and biomarker concentration-time profiles simulated by using the final PK/PD models will then be analyzed for correlations with clinical effects of the drug (response, toxicity*).* In the next step, clinical data, e.g. tumor size, can be incorporated in order to develop integrated 'PK/PD/outcome models'.
In conclusion, the development of semi-mechanistic PK/PD models based on biomarker data is feasible for targeted anticancer drugs. Future studies will reveal the potential to individualize therapy, e.g. by defining an optimal dosage regimen for each individual patient.

Reference:
Lindauer A et al. Clin Pharmacol Ther 2010;87:601-608

Key6-1

STABILIZING INACTIVE KINASE CONFORMATIONS WITH SMALL ORGANIC MOLECULES

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The complexity of kinase biology is controlled by layers of regulatory mechanisms involving different combinations of post-translational modifications, intramolecular contacts, and intermolecular interactions. Ultimately, these mechanisms achieve their effect by favoring particular conformations that promote or prevent the kinase domain from catalyzing protein phosphorylation. A more detailed understanding of the structural principles that regulate protein kinase activity allowed us to develop a novel fluorescence-based binding assay (FLiK) for the identification and optimization of inhibitors that stabilize enzymatically incompetent kinase conformations.

Key6-2

PLANT POLYKETIDE SYNTHASES IN THE BIOSYNTHESIS OF ACTIVE CONSTITUENTS

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Plants produce a huge array of natural products which display a range of biological activities. Plant constituents have long been exploited by humans as sources of drugs and continue to play an important role in drug discovery, either as the actual drug substances, semisynthetic derivatives or synthetic analogues. A major group of plant-derived natural products are polyketides which are formed by a family of homodimeric enzymes, called type III polyketide synthases (PKSs). A rare starter substrate for PKSs is benzoic acid. We detected and expressed in *E. coli* two plant type III PKSs that condense benzoyl-CoA with three malonyl-CoAs to form either a benzophenone or a biphenyl, thus referred to as benzophenone synthase and biphenyl synthase, respectively. Benzophenone metabolism, e.g. in *Hypericum* species, involves a number of active polyprenylated polycyclic compounds and biphenyl derivatives are defence metabolites of apple.

Reprogramming of biosynthetic pathways is an attractive approach of modifying the structures of natural products. Enzymes can be engineered to accept altered substrates and/or to produce new types of products. Enzymatic steps can be added or blocked. These metabolic engineering strategies have been successful in microorganisms but have not been widely explored to yield unnatural natural products from complex, lengthy plant pathways. Heterologous production of plant constituents in recombinant microorganisms is still often limited by the paucity of cloned genes involved.

We subjected benzophenone synthase to site-directed mutagenesis. The enzyme was converted by a single amino acid substitution in the active site cavity (Thr135Leu) into a functional phenylpyrone synthase, a novel type III PKS variant. The dramatic change in both product and substrate specificities was rationalized by homology modeling. A new pocket may be opened by the point mutation. Two isoenzymes of biphenyl synthase were found to prefer salicoyl-CoA as a starter substrate, leading to formation of 4-hydroxycoumarin, a precursor of dicoumarol. These findings provide the chance of generating new natural products in transgenic plants and derived tissue cultures that express engineered enzymes.

J. Biol. Chem. 2009, *284*, 30957; Plant Mol. Biol. 2009, *72*, 17

Key6-3

CHEMOENZYMATISCHE WIRKSTOFF-SYNTHESE

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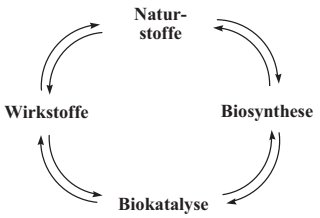
Naturstoffe bieten als privilegierte Strukturen einen erfolgversprechenden Einstieg in die Wirkstofffindung. Wir nutzen die durch die Biosynthese vorgezeigten Produktionsrouten in Kombination mit Diversitäts-orientierter Synthese zur Darstellung neuer potentieller Wirkstoffe.



Die Vielfalt der Naturstoffstrukturen stellt einen der Gründe für deren Eigenschaft als ‚privilegierte' Strukturen dar. Gleichzeitig ist dies aber auch von Nachteil bei dem Versuch ‚biomimetische' Syntheserouten hin zu definierten Zielmolekülen zu etablieren. Während die Natur auf oftmals rasch wechselnde Bedingungen flexible Antworten finden musste, dient die zielorientierte Synthese der selektiven Darstellung eines Produktes mit optimierten Eigenschaften für eine bestimmte Anwendung.
Von uns wird eine biomimetische Synthesestrategie basierend auf methodischen Untersuchungen der Biosynthese propagiert. Dies resultiert in einer chemo-enzymatischen Synthese einer Vielfalt von Produkten unter Verwendung biokatalytischer in-vitro und in-vivo Transformationen in Kombination mit selektiven nicht-enzymatischen Syntheseschritten.
Hiermit werden die Vorteile enzymatischer Synthesen, hohe katalytische Aktivität, ausgeprägte Selektivität und mögliche Übertragbarkeit in Ganzzell-Produktions-prozesse, mit denen der nicht-enzymatischen Methoden, breite Substrattoleranz und Produktvielfalt, in hervorragender Weise kombiniert. Im Endeffekt resultiert dies in einem Zugang zu einer Naturstoff-ähnlichen Strukturvielfalt mit einer hohen Wahrscheinlichkeit für biologische Aktivität.

Key7-1

BIG IS BEAUTIFUL – HESylation® AS AN EXAMPLE FOR DRUG-POLYMER CONJUGATES

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Drug delivery systems play a crucial role in optimising drug properties. Within the last decade polymers conjugated to pharmaceutically active molecules have emerged as one of the preferred tools in modern pharmaceutical development enabling the scientist to tailor the properties of the drug. Due to polymer conjugation the increase of the molar mass leads to reduced kidney excretion and results in a prolonged plasma half life of the drug. Furthermore, the shielding of the drug with the polymer may reduce the recognition of the immune system and its consequential clearance from the body. These effects have been demonstrated for several macromolecule classes, the most frequently used being poly ethylene glycol (PEG), albumin and poly carbohydrates like hydroxyethyl starch (HES).

HES products have been widely used as plasma volume expander in clinical practice for decades. They show an unprecedented safety record with daily administration exceeding 200g for adults. Their biodegradability is well known and there are no negative effects on the inflammatory system. Even after repeated infusions, HES in not accumulated in the blood plasma. Allergic reactions were found at 0.06% of patients and its immunogenicity was reported to be neglectible. The HESylation® Technology platform is based on the extensive expertise in the field of HES: a drug delivery technology linking HES derivatives to drug substances has been established by Fresenius Kabi, the world's largest producer of pharmaceutical grade HES.

Like PEGylation Fresenius´ HESylation® Technology allows the targeted modification of drugs by site-specific coupling. Besides plasma half life, HES-coupling frequently improves key parameters such as bioavailability, solubility, stability and safety parameters of the respective drug. A broad portfolio of process variants, coupling chemistries and specific chemical linkers have been developed to allow for a wide range of customised HES drug conjugates. At present, the most important application for the HESylation® Technology is seen in the area of biopharmaceuticals.

Key7-2

NEUE STRATEGIEN ZUR VERLÄNGERUNG DER HALBWERTSZEIT REKOMBINANTER PROTEINE

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Die therapeutische Anwendung von kleinen Proteintherapeutika ist oft durch die kurze Halbwertszeit limitiert. Strategien zur Verlängerung der Halbwertszeit rücken deshalb zunehmend in das Interesse der pharmazeutischen Forschung und Entwicklung. Dies beinhalten Strategien zur Vergrößerung des hydrodynamischen Radius, z.B. durch Konjugation hydrophiler Polymere und Kohlenhydrate, aber auch die Fusion an Plasmaproteine, wie z.B. Albumin, bzw. albuminbindende Strukturen. Neben einer Größenzunahme kann hierdurch auch ein Recycling über den neonatalen Fc-Rezeptor implementiert werden. Die Grundlagen als auch die Auswirkungen auf die Halbwertszeit verschiedener Strategien (PEGylierung, N-Glycosylierung, Fusion an humanes Serumalbumin, Fusion an eine Albuminbindedomäne von Protein G) werden anhand eines rekombinanten bispezifischen Antikörpermoleküls vergleichend dargestellt.

Key7-3

NEXT GENERATION SITE-SPECIFICALLY PEGYLATED FVIII FOR THE TREATMENT OF HEMOPHILIA A

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Hemophilia A is caused by deficiencies in coagulation factor VIII (FVIII) and is the most common hereditary coagulation disorder. FVIII circulates as a heterodimer composed of a heavy chain of approximately 200 kDa and a light chain of 80 kDa. It consists of the structural domains A1-A2-B-A3-C1-C2. The current treatment for hemophilia A involves intravenous injection of recombinant (rFVIII) or plasma-derived human FVIII. Injections of FVIII are either given on demand in response to a bleeding event or as a prophylactic therapy that is administered 2 to 4 times a week. The requirement for frequent injections is primarily due to the short circulating FVIII half-life of 12 to 14 hours in patients. In order to improve the therapeutic properties of FVIII for hemophilia patients, recombinant B-domain deleted human FVIII (FVIII-BDD) was modified at various positions by site specific PEGylation. The resulting PEGylated BDD muteins were evaluated for FVIII activity and plasma half-life. Activity assays showed that FVIII activity was retained following PEGylation. In vitro characterization was consistent with PEGylation occurring at the intended locations. Pharmacokinetic studies identified muteins with an increased plasma half-life. In bleeding models of hemophilic mice, PEGylated FVIII-BDD not only exhibited prolonged efficacy that is consistent with the improved pharmacokinetics but also showed efficacy in stopping acute bleeds comparable with that of unmodified rFVIII. In summary site-specifically PEGylated FVIII has the potential to be a long-acting prophylactic treatment while being fully efficacious for on-demand treatment for patients with hemophilia A.

Kurzvorträge
Pharmazeutische Technologie

T1-1

EMULSIFICATION (O/W) IN MICROCHANNEL GEOMETRIES FOR PHARMACEUTICAL SCREENING APPLICATIONS

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In pharmaceutical industry, emulsification processes are of high relevance for the handling of active ingredients. Typically very valuable agents are applied. These are on the one hand very expensive and on the other hand only exist in small quantities. To reduce both, expenditure of cost and time for the development of such pharmaceuticals during agent and formulation screening, a handling procedure has to be provided for these small quantities. Microsystem technologies offer several advantages concerning this aspect, such as small fluid volumes and precise control of process conditions. In the framework of the DFG research unit 856 mikroPART a microsystem for O/W emulsification was developed. The used formulation is Miglyol® 812 as dispersed phase and distilled water as continuous phase. To keep the droplets stable, 3 % Solutol® HS 15 is added to the continuous phase. The microsystem consists of one inlet for the disperse phase, at least one inlet for the continuous phase, a droplet formation zone in the middle and one outlet for the obtained emulsion. It consists of a structured top base made of poly(dimethlysiloxane) (PDMS) which is bonded to a glass bottom to attain a closed microfluidic device. This material combination allows microscopic observation during emulsification process because of the given transparency of both materials. The influence of the channel geometry on the droplet formation was analyzed according to the two known principles: T-junction and flow-focusing. For both cases the design and size of the droplet formation zone was modified: the inlet of the disperse phase at the T-junction and the nozzle geometry of the flow-focusing systems. For the later design the angle between the inlets of the two phases was varied. In general, acute angles lead to larger droplets. Besides the geometrical influence, the dependence between the volume flow ratio $Q_{continuous}/Q_{dispers}$ and the resulting droplet size was analyzed. With increased volume flow ratios, a decrease in droplet size can be attained. The presented microsystems generate well controllable, monodispersed emulsions. Depending on the volume flow and the microchannel geometry, oil droplets were generated with minimal diameters of 30 µm.

T1-2

USING MICRO HEAT EXCHANGERS FOR PHARMACEUTICAL APPLICATIONS

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[2]Institut für Pharmazeutische Technologie, TU Braunschweig

Micro-structured systems offer many advantages compared to macro-scale systems among which process intensification is the major one. Process intensification in heat transfer processes leads to the possibility of moving from batch to continuous processing mainly due to high heat and mass transfer coefficients. This effect is mainly caused by the very high surface to volume ratio. Another advantage for sensitive products is the low shear stress in the laminar regime and the short residence time. Additionally the hold-up of the apparatus is in the range of some milliliters.
A model batch process of crystallization of drug carrier lipid nanoparticles is used here to demonstrate some benefits and application possibilities of micro heat exchangers. Currently the crystallization of solid lipid nanoparticles is usually performed in a batchwise process under poorly defined cooling conditions, without much regard to the aspects of heat transfer and precise process control. In addition, these setups often only allow the application of low cooling rates. The use of high, well-defined cooling rates could offer very interesting new possibilities for the production of such drug carrier systems[1].
Using a micro heat exchanger device, high and well-defined cooling rates can be achieved for the continuous melt crystallization of solid lipid nanoparticles. During the crystallization of various solid lipid nanoparticle formulations it was found that some formulations lead to particle deposition and blocking of small passages in the micro heat exchanger. Obviously, specific interactions between the lipid nanoparticles and the surfaces of the device may occur and need to be considered in the design of the process as well as of the equipment. For formulations without deposition tendencies, reproducible experiments show that the continuous microfluidic process is applicable to produce solid lipid nanoparticles with constant product properties, like particle size and polymorphic form.

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T1-3

3D FLOW FIELD MEASUREMENTS IN COMPLEX MICROSYSTEMS

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State-of-the-art Microsystems for pharmaceutical applications have complex geometries which induce complex fluid motion. To validate the efficiency of these microsystems, the challenge of measuring the 3D flow field has to be overcome. Moreover, it is essential to characterize the mixing of different pharmaceutical components in Microsystems in order to validate their effectiveness. To solve both issues, a novel measurement technique to locate and track the motion of individual particles in a fluid volume (3D) has been developed at the UniBw München. This method has the capability of instantaneously determining the three-dimensional positions of tracer microparticles inside the flow when their image is captured by a digital camera. The basic principle of the technique consists of breaking the axis-symmetry of the optical system, introducing astigmatism to the digital recordings. This optical distortion gives the particle images an elliptical shape whose dimensions change as a function of their depth position. The astigmatism effect is introduced to the optical system by a cylindrical lens mounted directly in front of the camera's sensor, which is coupled to the output port of a state-of-the-art inverted microscope.
The accurate identification of the three-dimensional locations of tracer particles in a volume at consecutive instants of time, allows for the calculation of all three components of the flow velocity. The simplicity of this measurement technique makes it stand out over other known approaches that attempt to measure similar flow conditions. This innovative flow diagnostics method only requires one camera, takes advantage of the illuminated volume in its entirety, and has been validated with benchmark flows to prove its robustness and reliability.
On the other hand, the potential to instantaneously identify the three-dimensional position of tracer particles in a fluid volume allows for the reconstruction of the physical state of the seeded flow at a specific time. This is of particular importance when attempting to characterize the interaction between two fluids, as they would inside micro structures designed for efficient mixing of several species. By seeding one of the fluids to be mixed, which allows for the reconstruction of the volume that it occupies in the micro mixer, the interface with its non-seeded counterpart can be characterized precisely.

T1-4

ADHESION OF SOLID LIPID NANOPARTICLES ON POLYELECTROLYTE MULTILAYER COATED SURFACES

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In the production of nanoparticulate drug carriers in microsystems, the adhesion of particles to the surface causes a loss of active pharmaceutical ingredients (APIs) and affects the product quality (due to changes in flow and heat transfer conditions or due to cross-contamination). Being able to control particle adhesion and cleanability by adjusting the surface properties in such devices is therefore a crucial point.
Polyelectrolyte multilayers (PEMs) represent a versatile tool for surface modification. They can be assembled practically independent of the chemical nature of the substrate by adsorption from aqueous solution in a layer-by-layer dipping process and - by using a dynamic flow-through principle - even in capped microsystems.
In this study, the adhesion of solid lipid nanoparticles on PEM-coated stainless steel was investigated. The type of PEM and its surface charge were varied. Using PEM as a baselayer, poly(ethylene glycol) (PEG) was additionally incorporated by subsequent adsorption of a custom-made Poly(acrylic acid)-*graft*-PEG (PAA-g-PEG) copolymer from water.
Three nanoparticulate suspensions were tested: two consisting of a mixed matrix of hard fat and phospholipid (native and hydrated) stabilized by 1 % Macrogol-15-stearate and one consisting of a mixed matrix of carnauba wax and decyl oleate stabilized by 1 % Polysorbate 80.
The relative amount of adsorbed particles was investigated by highly sensitive Fourier transform infrared spectroscopy in attenuated total reflection mode (FTIR-ATR) in dependence on the environmental conditions at adsorption (room temperature / elevated temperature / temperature ramp).
Both enhancement and reduction of particle adhesion could be observed for PEM compared to bare stainless steel. In general, the PEGylated PEM showed least adhesion of particles. The cleanability of PEM and PEM/PAA-g-PEG coated stainless steel was examined using a cleaning procedure with ethanol. It was found to be highly effective for hard fat particles but ineffective for wax particles.

T2-1

BACTERIAL NANOCELLULOSE: INFLUENCE OF FREEZE-DRYING ON THE DELIVERY OF DRUGS

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Bacterial nanocellulose (BNC) is a hydrogel, which provides numerous advantages for the use as drug delivery system. Because of its biocompatibility and remarkable material properties it has already been described as well suited for various medical applications [1].
In the present study, freeze-drying of BNC hydrogels as a method to accomplish long-term storage and easy application was investigated with regard to influence on the loading and release of the model drug bovine serum albumin (BSA).
Comparative in vitro release experiments between never-dried and freeze-dried BNC samples were performed. Effects of protein concentration, incubation time, temperature, type of release medium, pH and pre-swelling on loading and release of BSA were investigated. Protein quantification was carried out by UV spectroscopy. Loading and release of BSA could be controlled by all varied parameters. Steady-state conditions of the release could be observed for all tested BNC gels after 24-48 hours. However, freeze-dried samples loaded and released a lower amount of BSA (70-80%) compared to never-dried samples which could be correlated to the changes in the three-dimensional network structure after freeze-drying. Nearly comparable release results for initial never-dried and freeze-dried samples were obtained by freeze-drying after BSA loading. In conclusion, freeze-drying is a suitable method to improve handling as well as storage stability of protein loaded BNC gels in pharmaceutical applications.

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T2-2

DRUG NANOPARTICLES PREPARED BY NOVEL COMBINATIVE PARTICLE SIZE REDUCTION TECHNIQUES

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The majority of new chemical entities (NCEs) suffer from undesirable physico-chemical and biopharmaceutical properties, leading to erratic drug absorption, food effects and low oral bioavailability. Particle size reduction is a practical approach to enhance the oral exposure of these poorly soluble drug molecules, particularly for those molecules with a dissolution rate dependent bioavailability. Top-down technologies as wet ball milling (WBM) or high pressure homogenization (HPH) are currently the most advanced technologies with several commercial products on the market. Both technologies utilize mostly micronized APIs in order to prevent a clogging of the machines. The particle size is further reduced by means of mechanical comminution caused by shear forces, impaction forces or cavitation forces. Long milling times, abrasion of milling beads and wearing of the machines are disadvantages of the standard top-down techniques.
A relatively new and smart way to produce nanosuspensions is the combination of two particles size reduction principles to improve the process effectiveness. In a first step the coarse starting material is modified using one bottom-up step and further processed into nanosuspensions by applying a top-down technique.
Currently different combinative technologies are employed. The first one is known as H42 process and can be described as combination of spray-drying (bottom up step) and high pressure homogenization. Another combinative technology is known as H96 process. This technology combines the modification of the starting material by means of freeze drying (bottom up step) with different top town technologies, such as WBM or HPH. Besides these two principles other combinative technologies exist, e.g. combinations of precipitation and HPH as well as combinations of WBM with HPH.
The work presented here gives a detailed overview of different novel combinative particle size reduction technologies. It will discuss the reasons for the improved particle size reduction effectiveness and how to optimize the particle size reduction effectiveness by selecting an appropriate technology and process conditions.

T2-3

SHEDDING LIGHT ON THE INTRACELLULAR PROCESSING OF REDUCTION SENSITIVE POLY(ETHYLENE IMINE) GENE CARRIERS

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Linear, low molecular weight (M_W) poly(ethylene imines) (PEIs) crosslinked with reduction sensitive disulfides to high M_W branched derivatives are highly efficient delivery agents for DNA as well as for small interfering RNA (siRNA). They do not share the cytotoxicity of their non-cleavably branched counterparts as they are intracellularly degraded into small less toxic fragments by cellular thiols like glutathione.[1] The exact location and time point of this reduction process are important as they determine if the nucleic acid reaches its destination before the carrier is degraded. Prematurely released nucleic acids would most likely have a signify-cantly lower effect on target cells. So far, however, this process is poorly understood.[2]
To gain a better understanding of those events we linked the reduction sensitive fluorescent probe BODIPY FL L-cystine to linear PEI (lPEI) and cystine cross-linked PEI (bPEI). In a reductive environment BODIPY FL L-cystine is cleaved (as well as bPEI) into two highly fluorescent monomers making it the ideal tool for fluorescence based method like flow cytometry. In preliminary experiments we investigated the degradation of our PEI/nucleic acid complexes by glutathione using fluorescence spectroscopy. We observed significant differences in the cleavage behavior depending on the polymer and the nucleic acid used. Linear PEI seems to form much stronger complexes with DNA than with siRNA preventing the disulfide cleavage or possibly the DNA release. For polyplexes made with branched PEI however siRNA behaves like DNA leading us to the conclusion that polyplex cleavage and nucleic acid release might not necessarily be associated. We also evaluated the intracellular processing of DNA polyplexes by flow cytometry. By influencing the cellular trafficking and disulfide cleavage of our model nano-complexes with agents like chloroquine or N-ethylmaleimide we provide evidence that polyplexes based on linear PEI are differently processed *in vitro* than poly-plexes made of branched PEI.
We conclude that our findings underline the importance of custom designed carriers for gene therapeutics depending on the nucleic acid to be delivered.

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T2-4

MODULAR TARGET NANOPARTICLES – DRUG CARRIERS FOR RADIATION THERAPY OF CANCER

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Target Nanoparticles can enforce radio- and chemotherapy of cancer in two ways: concentration of the drug in particles, and targeted localization of therapeutic material in the tumor. A further advantage is the selective uptake and delivery at cellular level by endocytosis. For radiotherapy the drug is an enhancer, which enforces the radiation induced cell inactivation with respect to untreated or healthy cells lacking the uptake. In chemotherapy and combined radio-chemotherapy this inactivates the malign cells by bio-chemical interaction, leading to a tumor growth stop.
Our therapeutic nanoparticles for enhanced radiotherapy are combined from lipids, stabilizing polymers and magnetic iron oxides. The particles of ~100 nm size carry an enhancer load of lanthanide chelates or boron as radiation absorption target and chemotherapeutics, e.g. cis-Platin, as proliferation inhibitors. A major object are target liposomes bearing modular surface signals for cellular uptake, and the drug load. A premature uptake of the therapeutic target nanoparticles is avoided by novel stealth lipids bearing a polyglycerol head. Other nanoparticles contain polymers and poly-ferrofluids for magnetic drug targeting MDT and release.The particles are characterized by spectroscopy, dynamic light scattering DLS, electron microscopy, neutron and synchrotron X-ray scattering and imaging, and magnetic characterization. The drug release from target nanoparticles is observed by nano-dissolution experiments. This reveals the drug release at body temperature after administration, and the formulation stability during preparation and storage. The biocompatibility and possible toxicity is investigated with cancer cell cultures of several lines of brain, lung, liver, colon and kidney cancer. In our kinetic EPN test cell cultures are used as tumor models. This distinguishes between toxic or radio-toxic action and the favorate cancer cell proliferation inhibition, which is equivalent to a tumor growth stop. As radiation sources for neutron capture therapy NCT we use the reactors TRIGA Mainz, and ILL Grenoble, for photon therapy tests the accelerators at radiooncology clinics Mainz, and the ESRF synchrotron Grenoble.

T3-1

A POLYMER AS SOLVENT AND SUSTAINED RELEASE EXCIPIENT FOR LIPOPHILIC DRUGS – HEXYLSUBSTITUTED POLY(LACTIDE)

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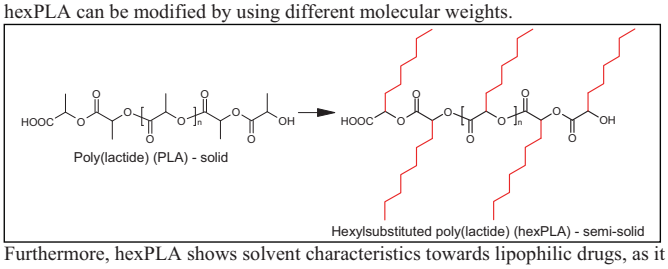
Hexylsubstituted poly(lactide) (hexPLA) is, like poly(lactide) (PLA) an entirely degradable polyester. While the *in vivo* degradability and good biocompatibility allow PLA to be widely used in the medical field for various applications, the pronounced burst-release and the solid aggregate state limit its use for parenteral release applications. These PLA formulations are not injectable on their own without the addition of plasticizers or the use of sophisticated application systems. In contrast, hexPLA is a viscous liquid at room temperature, allowing the direct incorporation of APIs and facilitating the injectability of the excipient without the need for additional organic solvents. The degradation and release profile of hexPLA can be modified by using different molecular weights.



Furthermore, hexPLA shows solvent characteristics towards lipophilic drugs, as it was illustrated with the dye Sudan III and the antipsychotic drug Haloperidol. Quantification by UV-spectroscopy of saturated solutions showed that the incorporation capacity of both substances was higher, the lower the molecular weight of the polymer was. Thus, formulations could be prepared with the same concentration of the API, but with different ratios of dissolved to suspended drug. A release study revealed, that the solution to suspension ratio had high influence on the burst-release characteristic. By using a drug solution, the initial burst can be avoided, which is of advantage for APIs with a narrow therapeutic window, as is Haloperidol. In conclusion, hexPLA can be used as a solvent and sustained release excipient for lipophilic drugs.

T3-2

DESIGNING POLYMER INTERLAYERS TO IMPROVE IMPLANT SURFACES

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Titanium and its alloys are widely used as implant materials due to their good biocompatibility and mechanical properties. With the help of polymeric coatings the implant surfaces can be adjusted in their properties according to the needs of application. Two different applications of this concept are highlighted here: Firstly, attaching signalling proteins like Bone Morphogenetic Proteins (BMP) to the surface employing a polymer interlayer aims at an improved osseointegration of the implant. Secondly the installation of antimicrobial polymers to the surface is targeted to prevent or at least retard adhesion of bacteria and the formation of biofilms. To address these issues we covalently attach polymers onto titanium oxide surfaces via photochemical grafting onto (1) or utilizing the surface activity of phosphonates (2), respectively. The coating results in ultra thin polymer films at the nanometer scale. For the covalent binding of BMP2 e.g. poly (4-vinylbenzyl) phosphonic acid diethylester-co-glycidyl methacrylate has been designed showing a high ability to bind the protein as determined by using an immunoassay. This immobilized protein retains some biological activity as tested by a cellular signaling system. Starting point of creating antimicrobial surfaces is the synthesis of copolymers e.g. poly (vinylbenzylphosphonate-co-hexylpyridinium) (3) combining the biocompatibility of phosphate groups with the antimicrobial effect of quarternized poly(4-vinylpyridinium).
Acknowledgement: This work was supported by the DFG as part of the SFB 599.

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T3-3

VEGF RELEASE FROM CA-/ZN-ALGINATE GELS AND THEIR PHYSICO CHEMICAL PROPERTIES

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The aim of this study was to develop an injectable drug delivery system for vascular endothelial growth factor (VEGF), based on alginate and *in situ* gelation. The desired route of administration requires the application of an ionotropic internal gelation method. $CaCO_3$ and/or $[ZnCO_3]_2 \cdot [Zn(OH)_2]_3$ represented the cross-linking salt, from which Ca^{2+} and Zn^{2+} are liberated by glucono-∂-lactone (GDL), to cross-link the alginate. Final pH value within the gel has to be considered carefully for reasons of biocompatibility and the influence on release kinetics of charged VEGF from alginate gel. pH value within the gel was determined by VIS spectroscopy after addition of phenol red and bromcresol green to the alginate solution prior to gelation. Results were verified by ESR spectroscopy. The obtained gels were found to be slightly acidic. To determine the influence of the gel structure on VEGF release, gels were neutralized after solidification. To identify the effect of cross-linking salt and alginate composition (guluronic (G) or mannuronic acid (M) content), these parameters were systematically varied and VEGF release determined by ELISA. In addition, alginate gels were characterized rheologically. Gels cross-linked only by Ca^{2+} showed strong burst release of VEGF. An increased Zn^{2+} content reduced the burst release. Gels cross-linked with more than 50 % Zn^{2+} showed a sustained VEGF release. Zn^{2+}, however, decreased the total amount of released VEGF. An increasing M content of the alginate, resulted in an reduced amount of VEGF release, whereas the shape of the release profile was maintained. In high G gels, even small Zn^{2+} contents considerably modified release profiles, whereas VEGF release from high M gels was less affected. Rheological properties were obtained by oscillating rheometry. Gels cross-linked by Ca^{2+} showed higher storage moduli and viscosities than gels cross-linked by Zn^{2+}. Mixture of $Ca^{2+}$and Zn^{2+} showed an intermediate storage modulus, observed for high G and high M gels. By selecting suitable combinations of cross-linking salts and types of alginate, VEGF release profiles can be adjusted to different needs.

T3-4

OPTIMIZED DEGRADATION AND MECHANICAL PROPERTIES OF POLYMER FILMS FOR SURGICAL ADHESION PREVENTION

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Thin polymeric films made from biodegradable polymers are a well established tool for the prevention of tissue adhesions after trauma or larger surgical procedures. They can be used after gynecological surgery as well as after heart surgery or to treat injuries of tendons by minimizing the undesirable growth of scar tissue within neighboring tissues. For all these application degradable polymer films made from poly(lactic acid) [PLA] have proven to be well suited, however, an improvement with respect to their *in-vivo* degradation and an easier surgical applicability is highly desirable.
In order to achieve this goal the ultrathin polymeric films were modified in different ways using excipients or copolymerization. In order to speed up the degradation dilactide - yielding lactic acid in water - and poly(ethylene glycol) [PEG] - enhancing the uptake of water - were added to the solvent cast polymer films. Both substances only provided a faster degradation (indicted by gel permeation chromatography) and softening (indicated by the glass transition) for a limited time (few weeks), since both excipients were lost due to their excellent solubility in the degradation medium. The alternative copolymerizations with PEG led to polymer films with increased wettability indicated by smaller water contact angles, and also an enhanced uptake of water during degradation. Copolymers were synthesized from PEGs with different molecular weights (1k to 20k Da) and with different geometries (4 arm and 8 arm) [1], resulting in ABA triblock copolymers in case of the linear PEGs or star-shaped polymers for the respective star PEGs with attached multiple PLA chains. The release of water soluble PEG was significantly reduced due to the copolymerization, leading to a longer persistence in the polymer films with all its beneficial effects on water uptake and film softness. During the degradation the enhanced water uptake as mediated by PEG lead to a faster film breakdown (< 4 weeks) with softer breakdown products due to the remaining softening effect of the PEG components.
It was successfully demonstrated that copolymerization of PLA with PEG can be used to prepare surgical adhesion barriers with faster degradability and softer degradation products, which are hopefully less harmful to the surrounding tissue.

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T4-1

THE SILVER - NANOLIPID - COMPLEX (sNLC): IN VIVO EFFICACY

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The silver-nanolipid-complex (sNLC) is a combination of microsilver particles (10µm) and nanostructured lipid carriers (NLC). At the end of 2008, by chance, it was found that this combination can be active against light to servere medium atopic dermatits. Until now the mechanism of action remains not clear and is thus aspect of many research activities. The proposed mechanism of action is the release of positively charged silver ions from the silver microparticles, which absorb onto the surface of the negatively charged nanoparticles forming silver ion coated NLC (sNLC). NLC are known to poccess increased adhesiveness, therefore in theory the retention time of the silver ions bound to the NLC is longer at the desired site of action (e.g. skin or bacteria surfaces), leading to an improved activity than silver ions allone.
The adsorption of silver ions onto the surface of the NLC could be shown by applying Asymmetric-Flow Field Flow Fractionation coupled with Multi Angle Light Scattering (AF4-MALS). Other frequently applied techniques e.g. dynamic light scattering were not suitable to prove this. The in vivo efficacy was investigated by studying a) the antibacterial activity (e.g. using the well agar diffusion method and broth dilution method), b) the anti-inflammatory activity and c) the erythema score in a atopic dermatitis mouse model. In all studies not only an additional, but synergistic effect was found, when silver was combined with NLC. The data suggest that sNLC might be a novel and effective therapy concept for the treatment of atopic dermatitis without adverse side effects. Further studies are ongoing.

T4-2

FINITE DOSE SKIN PENETRATION - EXPERIMENT AND SIMULATION

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By definition, for finite dose skin absorption experiments a dose of less than 10 µl/cm² or 10 mg/cm² is applied to the skin surface [1]. In this study in vitro finite dose skin penetration experiments were established and the results were compared to simulations of a 2D diffusion model in order to show, whether this model developed for infinite dose can correctly predict finite dose skin absorption.
The experiments were performed using human abdominal full-thickness skin in a Franz diffusion cell. A finite volume of 5-8 µl/cm² of the model drug flufenamic acid in aqueous solution was applied.
For simulating the experiments, we extended the functionality of a previously established 2D diffusion model, which had been developed and validated for infinite dosing [2,3]. The model is based on the anatomical structure of the skin and uses only physicochemical input parameters.
Simulated and experimental concentration-depth profiles for the SC and the deeper skin layers using the same physicochemical input values as in the infinite dose experiments correlated reasonably.
As expected, for finite dose experiments, the drug amount in the donor decreased both in the simulation and the experiment. However, this effect was more pronounced for the experimental data set resulting in high drug amounts in the SC already after 15 minutes. This finding does not correlate well with the simulation.
One reason might be that the outer, loosely packed corneocyte layer of the SC, the stratum disjunctum, does not exhibit a strong barrier function and might have quickly soaked up the donor solution. Future work comprises the inclusion of this mechanism in the model framework.

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T4-3

THE PERMEATION STUDY OF TERBINAFINE HCL FROM POLOXAMER 407 BASED THERMOGELLING FORMULATIONS ACROSS ISOLATED HUMAN STRATUM CORNEUM

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The permeation of drug across skin is determined by two main steps, i.e. the interaction of vehicle with the stratum corneum and the drug thermodynamic activity within the formulation. The vehicle can control the drug release and in some extent modifies the barrier properties of the stratum corneum.
In this study, 1% terbinafine HCl, a lipophilic antifungal drug with log P 3.3 and aqueous solubility of 0.7% was incorporated into semisolid poloxamer 407 based thermogelling formulations and the permeations across isolated human stratum corneum were examined. The formulation was composed of the mixtures of poloxamer 407: middle chain triglycerides Miglyol® 812N (4:1), isopropyl alcohol: dimethyl isosorbide (1:1) and water. The mixture of poloxamer 407: Miglyol® 812N was employed from 25-50%, meanwhile the mixture of isopropyl alcohol: dimethyl isosorbide (penetration enhancers) was from 12-40%. The formulation was manufactured using Cito unguator 2000 Konietzko GmbH at the speed of 1450 rpm for 1.5 min. The marketed product Lamisil® Creme and a 20% poloxamer hydrogel containing 1% drug were tested as well. The complex viscosities of the formulations were measured using a controlled stress rheometer CVO 50 from Bohlin.
The results showed that high contents of poloxamer and water were responsible for the high viscosity of the formulations; meanwhile the penetration enhancers decreased the viscosity. There was a good agreement between the complex viscosity and the reciprocal drug flux and this was also the case for the drug accumulated amount in the stratum corneum after 48 h of permeation. The apparent flux increase along with the increase in enhancers contents was rather due to the decrease in the viscosity of the formulation. All flux values were higher compared to those from Lamisil® Creme, except for the formulation with more than 40% poloxamer content. The formulations with viscosities about the same magnitude showed about equal flux values, showing that variation in compositions was not affecting the amount of permeated drug across the skin. Furthermore, viscosity could be a predictive tool in estimating the drug flux from this formulation.

T4-4

INFLUENCE OF IBUPROFEN CONTENT ON THE RHEOLOGICAL AND THERMAL BEHAVIOR OF AN ACRYLIC PRESSURE SENSITIVE ADHESIVE

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Adhesion of transdermal systems to the skin is a critical factor directly related to cutaneous drug penetration and thus therapeutic effect. It is well known that the viscoelastic behavior plays a critical role in the performance of pressure sensitive adhesive (PSA) products and bonds. In the present study the change of the adhesion properties of DuroTak®-387-2051 (Henkel), a solvent-based acrylic PSA, is compared to the rheological and thermal behavior at increasing ibuprofen content.
Samples of DuroTak®-387-2051 with increasing ibuprofen content were prepared for rheological analysis by lamination multiple layers of 0.2 mm dry adhesive film to achieve a final thickness of about 1 mm. The samples were cut into discs of 25 mm diameter each. Temperature sweep and frequency sweep experiments were performed on a Rheometrics Dynamic Analyzer II (Rheometrics) equipped with a 25 mm parallel plate geometry and a convection oven. A frequency sweep was run at +32 °C followed by a combined temperature frequency sweep from -60 to +200 ºC (5 °C steps) at 0.1 to 100 rad/s. Linear viscoelastic behavior was confirmed by strain test at 100 rad/s. DSC measurements were done using a DSC7 (Perkin Elmer) with a heating rate of 10 K/min in a temperature range between -100 and 130 °C.
Tan δ curves were obtained from frequency-sweeps as well as combined temperature-frequency-sweeps. The latter were displayed in 3D plots showing a decrease of the dynamic glass transition temperature (Tg) with increasing drug content at all investigated frequencies. The minimum of the tan δ curves of samples with increasing drug content was found to be shifted to higher values.
Frequency sweep measurements of drug loaded samples at 32 °C also showed an increase in tan δ for all investigated frequencies. This indicates that the addition of ibuprofen to DuroTak®-387-2051 results in a loss of shear resistance and an increase in tack. The decrease in Tg and thus plasticization could be confirmed by DSC measurements.

T5-1

END POINT CONTROL OF AN ACTIVE COATING PROCESS BY RAMAN SPECTROSCOPY

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In the formulation of solid dosage forms film coating represents an important unit operation which can provide different functions like taste masking, product identification and protective layering. Active coating is a specific application where the active ingredient is comprised in the coating layer. Active coating is a challenging operation regarding the achievement of desired amount of coating and coating uniformity. In order to guarantee the quality of such dosage forms it is desirable to develop tools that are able to monitor the coating operation and to determine the end point and the coating uniformity, respectively.

The model drug diprophylline was coated on placebo tablets and a multivariate quantitative calibration was developed using tablets collected at different stages of coating and increasing amount of API from a small-scale pan coater. The Raman spectral measurements were correlated with the amount of coated active ingredient at each time point by using PLS. Afterwards the developed model was validated in agreement with ICH guideline Q2 whereby the focus was the transfer to real time monitoring in order to demonstrate the suitability of Raman spectroscopy as PAT tool for in-line quantitative monitoring of active coating.
Typical validation characteristics for assay procedures like accuracy, specificity, precision, range, and linearity were examined. Additionally the detection limit and quantitation limit were included in the investigation in order to assign the area in which quantitative inline monitoring of active coating is possible. Furthermore, the repeatability should be assessed using samples, which cover the specified range for the procedure.
After validation the developed model was used successfully to monitor the progress of coating in a laboratory film coater BFC 5 by the inline measurements and to determine the end point of active coating. Finally the model developed on the lab scale pan coater with a batch size of 3.5 kg could be transferred in a scale up experiment to a pilot scale coater with a batch size of 30 kg.

T5-2

HOT MELT EXTRUSION OF LOW MOLECULAR WEIGHT CRYSTALLINE MATERIALS

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Hot melt extrusion is a well established technology which is mainly used for amorphous compounds. The melt viscosities of low molecular weight crystalline materials are frequently too low to produce mechanically stable extrudates from the extrusion die. In order to overcome this issue, a new technology was proposed in which the extrudate crystallized rapidly at the extrusion die.
In this study, a new extrusion die was developed and validated to extrude melts of low molecular substances, such as mannitol, using a lab scale extruder (Leistritz, Mikro GL27-28D). The main problem which had to be mitigated was a temperature gradient across the die which led to crystallization within the die and subsequent clogging.
The extrusion die was varied systematically by insulating it as well as by adding multiple heating devices to various positions on the die. The temperature gradient across the die and temperature fluctuation were monitored by temperature gauges added to critical parts of the die. Finally, the temperature gradient was decreased from 21 to 7°C while the temperature fluctuations were reduced from 14 to 1.5°C. Using this optimized die configuration, it was possible to extrude a powder mixture of 90 % mannitol and 10 % griseofulvin. The extrudate had an adequate shape, and the drug and the excipient were in the crystalline state. The dissolution rate of the drug from the extrudates was much higher than from the physical mixture.
In conclusion, the extrusion of crystalline materials is possible using a twin screw extruder with a modified die. Since the end product is crystalline, the stability issues common to amorphous systems can be avoided.

T5-3

POROUS CARRIERS AS A TARGET FOR DRUG LOADING BY SUPERCRITICAL FLUID TECHNOLOGY USING AN OPEN OR ENVIRONMENT FRIENDLY CLOSED LOOP SYSTEM

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Purpose:
Development of a system to load sparingly soluble drugs into preformulated carriers with tailored dissolution properties by the use of environment-friendly supercritical fluid technology.
Methods:
Loading of a model drug (cumarin) was performed using controlled particle deposition from a supercritical solution. In a 2.1 l loading chamber 3.0 g cumarin was incubated with supercritical CO_2 for 3.5 h (15 MPa; 40 °C; 2 h / 30 MPa; 60 °C; 1,5 h). Afterwards the system was relaxed adiabatically without falling below the critical temperature, either in a closed loop system with recycling of the CO_2 and drug or in an open system without recycling.
Porous carriers with different dissolution properties were formed from Avicel PH 102®, ethylcellulose, Eudragit RS PO® and corn starch. Glass frits and cylindric lump sugar were used for comparison. The carrier porosity was determined (gas-comparison-pyknometry) before loading and the drug content (UV-VIS at 280nm) and dissolution (Stricker model, pH7.4) after loading. Drug particle distribution was observed by fluorescence microscopy (350/420nm).
Results:
The porosity of the carriers varied according to the carrier composition between 32.37±2.22 and 71.22±2.05 (%±SD,n=5-20). Fluorescence microscopy showed homogenous allocation of small deposited particles across the tablet. Due to the different loading conditions (i.e. variations of T, p, t) the drug content varied depending on the tablet porosity, material and working conditions. Dissolution showed modulated release properties according to the carrier system used.
Conclusion:
With the controlled particle deposition method (CPD) employing supercritical CO_2, we successfully loaded monolithic porous carriers with a model drug. Our closed loop technology presented here enables the recycling of both, CO_2 and the used drug, offering an interesting way to handle highly potent or toxic drugs. The final products show modified drug dissolution properties, as intended.

VARIOUS FORMULATION APPROACHES TO IMPROVE DRUG RELEASE FROM A FIXED DOSE COMBINATION PRODUCT

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In the present study we wanted to improve the dissolution behaviour of two BCS class II compounds, glimepiride, a weakly acidic drug and pioglitazone, a weak base, in a fixed dose combination product. Two different formulation approaches were used for this purpose. The first approach was an inclusion complex of each of the drugs with hydroxy-propyl-ß-cyclodextrin (HP-ß-CD) and the other one was a mixture of solid dispersions of each compound with Soluplus®, a recently marketed copolymer. The main objective was to obtain formulations that show a dissolution behaviour superior to that of each of the pure drugs and also to a marketed fixed dose combination.

A freeze drying procedure was used to prepare the inclusion complexes of both compounds and HP-ß-CD as well as the solid dispersions with Soluplus®. Formulations were then subject to thermal analysis, solubility and dissolution tests. To elucidate, if the dissolution performance of our new formulations is superior to that of the marketed product, solubility- and dissolution tests were performed in two test fluids simulating conditions in the stomach and the upper small intestine.

DSC spectra of the complex formulations indicated that true inclusion complexes and amorphous solid dispersions were obtained. Results of the solubility experiments showed a significant increase of glimepiride and pioglitazone aqueous solubility. Dissolution performance of both fixed dose combination formulations was superior to that of the pure drugs and the marketed formulation under gastric and small intestinal conditions.

Based on their *in vitro* performance, we assume that the *in vivo* behaviour of our formulations might be superior to that of the marketed formulation. This assumption and the applicability of these formulation approaches to other combinations of weakly basic and a weakly acidic BCS class II drugs needs will be proved in future experiments.

Kurzvorträge Pharmazeutische Biologie

B1-1

NEW ANTIBIOTICS FROM CYANOBACTERIA

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In the last decades screening programs revealed that cyanobacteria are rich sources of new active structures with potential as new pharmaceuticals or lead structures, nevertheless the rich resources of German lakes, the Baltic Sea and Asian countries are hardly screened so far. Due to recent development in bacterial resistance and the increasing incidence of MRSA-strains worldwide we focused on screening of antibiotic activity of such hardly tested cyanobacterial strains and isolation and structural elucidation of the active substances.
Laboratory cultures were established and biomass as well as cultivation medium was extracted with solvents of different polarity. Extracts have been tested in agar-plate diffusion assay for antibacterial and antifungal activity. Bioassay-guided isolation of the active compounds was done by column chromatography including HPLC. Structure was elucidated by analysis of ESI-MS-MS, ESI-TOF-MS, 1D (^{1}H and ^{13}C) and 2D (COSY, TOCSY, ROESY, NOESY, HMQC and HMBC) NMR spectra and amino acid analyses.
Separation of the n-hexane extract of *Limnothrix redekei* HUB 051 (Müggelsee, Germany) resulted in the identification of three unsaturated fatty acids, α-linolenic acid, coriolic acid and α-dimorphecolic acid showing antimicrobial activity in vitro. Separation of the methanol extract of the freshwater strain *Lyngbya* sp. resulted in the identification of four novel antibacterial active undecapeptins, lyngbyazothrins A-D. From methanol extracts of different filamentous cyanobacterial strains collected from lakes or acidic soils of rice, cotton and coffee fields in Vietnam several compounds with antibacterial activity have been isolated e.g. daklakapeptin, fluorensadiol, hapalindols, ambiguine isonitrils as well as carbamidocyclophanes, variably chlorinated paracyclophanes presented MIC values between 0.04 to 0.1 µmol against *Staphylococcus aureus*.
Our first results show that so far hardly investigated cyanobacteria from the Baltic Sea, German lakes and from Asian countries are sources of structural new active compounds with potential therapeutically value. Culture optimization to enhance the yields of the active compounds is in progress.

B1-2

CHANGING A MUTANT'S MIND

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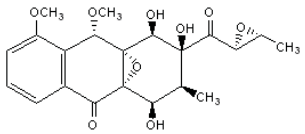
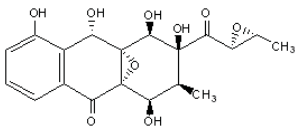
In 1998 Mensacarcin, a cytostactic substance with activity against many tumor cell lines, was isolated from *Streptomyces sp.* Gö C4/4. The anticancer potency of this new drug is similar to that of the clinically used doxorubicin[1]. The presence of two epoxy moieties in the molecule makes further investigations on the biosynthesis interesting, since these moieties are scarce in secondary metabolites of Streptomycetes.



Mensacarcin



Didesmethylmensacarcin

A gene cluster for the biosynthesis was identified, cloned and sequenced. The heterologous expression of this cluster in *Streptomyces albus* (*S. albus*) led to the production of the non-methylated derivative Didesmethylmensacarcin[2].
To elucidate the biosynthetic pathway of Didesmethylmensacarcin five oxygenases and four genes with currently unknown functions were investigated by Red/ET-mediated deletion and subsequent heterologous expression in *S. albus*. Unfortunately, the amounts of the new intermediates produced by these strains were very low.
By overexpression of the pathway specific regulatory gene *mnsR1* in the mutated strains the amount of the produced intermediates was drastically increased.
The chemical structure of these new compounds will be elucidated.

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[1] M. Arnold, *PhD thesis, University of Göttingen*, 2002
[2] A. Linnenbrink, *PhD thesis, University of Freiburg*, 2008

B1-3

TRADITIONAL CHINESE MEDICAL PLANTS INHIBIT ACETYL-CHOLINESTERASE, A KNOWN ALZHEIMER TARGET

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Inhibition of acetylcholinesterase (AChE) is a common treatment for early stages of the most general form of dementia, Alzheimer's Disease (AD). When AChE is inhibited, more acetylcholine is available resulting in an improvement of cognitive function. Although the therapy with AChE inhibitors (AChEI) is considered to be only symptomatic, these medications are still the first choice for treating AD patients in early stages of the disease.
In this study, methanolic, dichloromethane and aqueous crude extracts from 84 Traditional Chinese Medical (TCM) plants were tested for *in vitro* anti-acetylcholinesterase activity based on Ellman's colorimetric assay. Identity of the plants was assured by DNA barcoding. Thin Layer Chromatography (TLC) and mass spectrometry (MS) were performed to gain a first insight into the chemical compounds of the TCM plant extracts used.
Five TCM plants showed a notable inhibitory activity of AChE. Extracts from *Coptis* spp., *Capsella bursa-pastoris*, *Mahonia bealei*, *Phellodendron* spp., and *Polygonum multiflorum* exhibited a distinctive AChE inhibition with *Mahonia bealei* and *Phellodendron* spp. featuring this inhibitory activity in all three extracts. Two of these TCM extracts showed a stronger AchE inhibition than the already known AChEI galanthamine (EC_{50} = 4.33 µg/ml) with EC_{50} values ranging from 0.031 µg/ml (methanolic extract of *Coptis* spp.) to 2.5 µg/ml (aqueous extract of *Coptis* spp.*)*.
These findings suggest that Traditional Chinese Medical plants represent an important source of natural compounds that affect the activity of AChE, which might be interesting drug candidates to slow down the progression of AD.

IMMUNOFLUORESCENCE LOCALIZATION OF POLYKETIDE SYNTHASES IN THE MEDICINAL PLANT *HYPERICUM PERFORATUM*

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Extracts from *Hypericum perforatum* (St. John's wort; Clusiaceae) are widely used as antidepressants for the treatment of mild to moderate episodes. The medicinal plant is characterized by the presence of different types of secretory tissue including translucent glands, black nodules and secretory canals. *Hypericum* species are attractive experimental systems for studying the biosynthesis of a diversity of aromatic polyketides. Two type III polyketide synthases (PKSs) involved are benzophenone synthase (BPS) and chalcone synthase (CHS), for which cDNAs had been cloned and characterized. The enzymes were subjected to immunochemical studies and their distribution in *H. perforatum* was analyzed using immunofluorescence localization. Both enzymes were heterologously expressed in *E. coli* as His_6-tagged proteins and GST-fusion proteins. Polyclonal antibodies were raised against the His_6-tagged PKSs in rabbits and the IgG fractions were isolated. The specificity of the antibodies was examined using immunoblotting and immunotitration techniques. Protein extracts from various *H. perforatum* organs were subjected to SDS-PAGE and immunoblotting. BPS was mainly immunodetected in middle-aged fruits. CHS was detected in young leaves and flower buds. The tissue-specific localization of BPS and CHS was studied with *H. perforatum* organs using the immunofluorescence technique and confocal laser scanning microscopy. BPS was expressed to a low extent in mesophyll cells of young leaves and strongly expressed in the glandular cells of large translucent glands present inside the leaves. In roots, BPS was located in the cortex cells. In floral parts, as far as studied, BPS was found in the secretory tissue of sepals of young buds and in middle-aged fruits. In addition, seeds present in the middle-aged fruits contained BPS. CHS was strongly expressed in the mesophyll cells of young leaves and was not present in glands. Nor was the enzyme observed in roots. In floral parts, as far as studied, CHS is located in the mesophyll of sepals of young buds.

Kurzvorträge Pharmazeutische Chemie

C1-1

4-CYANO-1-OXO-β-CARBOLINES AS INHIBITORS OF PIM KINASES

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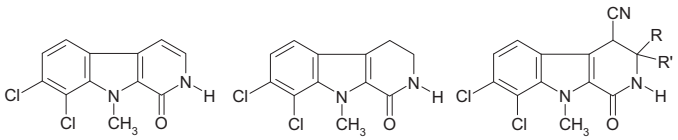
The 1-oxo-β-carboline alkaloid bauerine C (**1**) having some unique structural elements (dichlorophenyl moiety, *N*-methyl indole) was isolated from the blue-green alga *Dichotrix baueriana* showed antiviral and cytotoxic activities in preliminary screenings.
We worked out the first total synthesis of this alkaloid and found that bauerine C as and its dihydro derivative **2** have inhibitory activity against a broad range of kinases.

In order to improve the solubility properties and the selectivity against kinases, polar substituents were introduced into the molecule. For this purpose we worked out a new method for anellation of the lactam ring bearing a cyano group at C-4, as well as variable substituents (including spiro rings) at C-3.

Spiropiperidine analgues **3** of bauerine C showed potent and selective inhibition of PIM kinases and cytotoxic effects in screenings on tumor cell lines.



1 **2** **3**

C1-2

SYNTHESIS AND ANTIPLASMODIAL ACTIVITY OF REVERSE FOSMIDOMYCIN ANALOGS

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Inhibition of enzymes involved in the non-mevalonate pathway of isoprenoid biosynthesis represents a promising strategy for the development of novel antimalarials [1-3]. A series of reverse hydroxamate-based Fosmidomycin analogs was synthesized and evaluated for their inhibitory activity against the recombinant DXRs of *E. coli* and *P. falciparum* as well as for their antiplasmodial activity and cytotoxicity. The most active derivative inhibits the target enzyme 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) in the low nanomolar range and is devoid of cytotoxic effects on human MRC-5 cells.

Fosmidomycin (1): R = H
FR900098 (2): R = CH_3

Target compounds
R = H, Alkyl

[1] Jomaa, H. et al., *Science* **1999**, *285,* 1573. [2] Kurz, T.; Geffken, D.; Kaula, U. (BioAgency AG), DE 10356410 and WO 2005048715, **2005**. [3] Ruangweerayut, R. et al., *Malaria J.* **2008**, *7*, 225.

C1-3

SULFAMOYL BENZAMIDINES AS ARGININE MIMETICS: INHIBITION OF TRYPSIN-LIKE SERINE PROTEASES AND ACTIVE-SITE MAPPING

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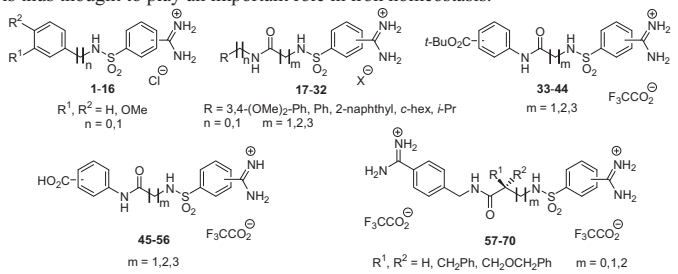
The substrate specificity of trypsin-like serine proteases is largely determined by an aspartate pointing to a cleavage specificity for arginine at P1 position. Substances with the benzamidine functionality as arginine mimetic are expected as potential inhibitors.[1] We prepared an extended series of *meta*- and *para*-sulfamoyl benzamidines and investigated the scope and the limitations in terms of the inhibition of trypsin, thrombin and the typ II transmembrane serine protease matriptase-2.[2,3] Matriptase-2 suppresses the transcription of the *Hamp* gene encoding hepcidin and is thus thought to play an important role in iron homeostasis.[4, 5]



	bovine trypsin K_i (μM)	human thrombin K_i (μM)	human matriptase-2 K_i (μM)
1-16	>30	>70	>80
17-32	4-40	>7	>50
33-44	7-60	7-200	>7
45-56	>20	>20	>5
57-70	0.1-9	0.5-40	8-40

[1] Peterlin-Mašič, L. *Curr. Med. Chem.* **2006**, *13*, 3627. [2] Velasco et al. *J. Biol. Chem*. **2002**, *277*, 37637. [3] Stirnberg, M. et al. *Biochem. J.* in press. [4] Silvestri et al. *Cell Metab*. **2008**, *8*, 502. [5] Finberg et al. *Nat. Genet.* **2008**, *40*, 569.

C1-4

SPIROCYCLIC σ RECEPTOR LIGANDS: EXPLORING HYDROPHOBIC POCKETS BY ARLYATION OF ANNULATED THIOPHENES

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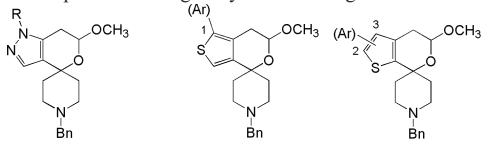
σ Receptors are well established as a receptor family with its own binding profile and a characteristic distribution in the CNS as well as in endocrine, immune and some peripheral tissues. Today at least two σ receptor subtypes have been identified which are termed σ_1 and σ_2 receptor. Modulation of σ_1 receptor activity offers some potential for the treatment of acute and chronic neurological disorders. Furthermore, some human tumor cell lines are able to express a large number of σ_1 and/or σ_2 receptors. Consequently σ_1 (and σ_2) receptor ligands may be used for diagnosis and therapy of cancer.
Our aim is to develop novel compounds with high σ_1 receptor affinity and high selectivity over the σ_2 subtype as well as other relevant receptors in the CNS. Recently we have shown that the substituent in position 1 of spirocyclic pyrazoles **1** influences considerably the σ_1 affinity. The spirocyclic pyrazole **1b** with a phenyl moiety in position 1 (R = Ph, K_i = 1.5 nM) is almost 15-fold more active than the corresponding methyl derivative **1a** (R = CH_3, K_i = 21 nM) [1]. Replacement of the pyrazole ring by a thiophene ring led to the very potent σ_1 ligand **2a** (Ar = H, K_i = 0.22 nM). So the idea came up to combine the high affinity thiophene substructure with an additional aryl substituent in position 1 (**2**), 2 (**3**) or 3 (**3**).
In the talk we report on the synthesis and the pharmacological properties of the arylated thiophene derivatives. The non-activated spirocyclic thiophenes **2** and **3** were regioselectively arylated in α- or β-position in presence of Pd-catalysts [2]. Finally the σ_1 and σ_2 receptor affinities of the synthesized σ receptor ligands were investigated in competitive binding assays with radioligands.



1 **2** **3**

[1] Schläger, T.; Dissertation **2008,** Münster [2] Yanagisawa, S.; Ueda, K.; Sekizawa, H.; Itami, K., *J.Am.Chem.Soc.* **2009**, *131*, 14622-14623
Financial support by the IRTG Münster/Nagoya (DFG) is gratefully acknowledged.

C2-1

CELLCULTURE STUDIES OF NOVEL CATIONIC LIPOSOMES USED AS NON-VIRAL VECTORS FOR GENE DELIVERY

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Martin Luther University Halle Wittenberg, Institute of Pharmacy

Gene therapy provides novel strategies for the treatment of acquired and inherited diseases, e.g. severe combined immune deficiency, cystic fibrosis and Parkinson's disease and it presents an alternative method to traditional chemotherapy against cancer. The principle of gene therapy is based on substitution, inhibition or addition of gene functions.
For application of genetic material it requires efficient vectors that protect nucleic acid against degradation and deliver it to target cells. These vehicles can be generally divided into two categories, viral and non-viral ones. Non-viral gene vectors such as cationic liposomes have several advantages compared with their viral counterparts, including low immune response, the ability to transfer large DNA molecules and they are easy to produce in large scale of similar quality. However, the low gene transfection efficiency is still the major disadvantage of these gene delivery systems.
Novel cationic lipids based on α-branched fatty acids have been synthesized in our group. To observe the structure-activity relationship the substances were modified, e.g. different chain length of the backbone or spacer and altered positive charged head group. We had combined them with diverse neutral helper lipids to form stable liposomes able to bind the chosen plasmid DNA encoding pCMV Sport β-Gal to form the lipoplex.
Cellular uptake and toxicity of these formulations were tested on different cell lines with serum-containing and serum-free medium. To get information about the transfection efficiency the β-galactosidase activity was measured using an ONPG assay. Cell viability was determined using a MTT assay.

C2-2

HYPHENATED BIOAFFINITY SCREENING – THE INTEGRATED SCREENING OF COMPLEX MIXTURES

Giera, M.[1], de Vlieger J.[1], Falck D.[1], Lingeman H.[1], Kool J.[1], Irth H.[1], Niessen W.M.A.[1]
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In today's drug discovery processes new approaches like metabolic conversions with biologically modified enzymes, or combinatorial chemistry approaches become more and more important to discover the chemical space around lead substances. The so generated substance mixtures require sophisticated separation and bioaffinity screening protocols in order to identify active substances within the generated mixtures. One possibility to face this challenge are so called high resolution screening systems (HRS), combining separation sciences, bioaffinity screening and mass spectrometry in a single platform. This combination leads to the simultaneous separation, bioaffinity determination and identification of active substances from crude mixtures. In this lecture we will discuss the principle of HRS, its benefits and drawbacks on basis of the successful development and implication of different target examples, including the estrogen receptors alpha and beta, the p38 MAP Kinase, as well as a screening approach for antibacterial substances. The development of an online HRS assay for the drug target MAP Kinase p38 alpha based on fluorescence enhancement and its application to screen for bioactive compounds generated by means of metabolic or chemical modifications will be discussed in detail.

C2-3

LIPID-BASED GENE VECTORS FOR VCAM-1 – KNOCKDOWN IN ENDOTHELIAL CELLS

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Besides its function in blood pressure regulation or hemostasis the vascular endothelium plays a crucial role in inflammatory reactions. Activated endothelial cells express inflammatory specific surface receptors like vascular cell adhesion molecule-1 (VCAM-1) which initiates binding and subsequent migration of leukocytes into the tissue and thus promotes inflammatory processes. Therefore, the restriction of VCAM-1 function could be a possibility in the therapy of autoimmune diseases. As one option the transfection of endothelial cells with α-VCAM-directed shRNA could lead to post-transcriptional downregulation of VCAM-1.

The aim of this work was to combine vascular targeting and gene manipulation using lipid-based gene vectors as an antiinflammatory approach.
We could show that the transfection of α-VCAM-shRNA into bEnd.3 endothelial cells induced an approx. 50% reduction of VCAM-1 expression levels compared to wild-type cells after stimulation with TNF-α. As a functional consequence reduced adhesion of VCAM-1 binding B16F10 melanoma cells to transfected bEnd.3 was determined applying a microscopic cell binding assay under flow conditions.
Based on these results we encapsulated α-VCAM-shRNA into liposomes which were applicable for systemic administration. Dual asymmetric centrifugation [1] provided small homogeneous liposomes with high DNA entrapment rates. Although the DNA remained intact during manufacturing procedure no down-regulation could be observed after incubation of liposomes to bEnd.3 cells. Alternatively, we investigated stabilized lipid-based particles (SPLP) as gene vectors which consist of a lipid-complexed single plasmid entrapped within a lipid bilayer [2]. The preparation by detergent-dialysis resulted in serum-stable vesicles of small and well-defined size and yielded high DNA encapsulation rates. Preliminary transfection experiments applying a GFP-encoding plasmid revealed SPLP to be a promising approach for vascular gene therapy.

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C2-4

PLASMA-LIQUID-INTERACTIONS: CHEMISTRY AND ANTIMICROBIAL EFFECTS

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Biomedical application of physical plasma is a growing field of research and development. Generally, biological plasma effects are mediated by liquid environments. Physical plasma treatment of liquid by a dielectric barrier discharge (DBD) under atmospheric air conditions resulted in microorganism inactivation accompanied by acidification as well as generation of nitrate (NO_3^-), nitrite (NO_2^-) and hydrogen peroxide (H_2O_2). These detected compounds resulted of complex plasma-liquid-interactions on the plasma/gas-liquid-interface and diffused from this interface into deeper layers of the liquid. To clarify possible mechanisms of reactive species generation as well as of microorganism inactivation in plasma-treated liquid, the interface between plasma and liquid phase was analyzed by Fourier transformed infrared spectroscopy (FT-IR) and optical emission spectroscopy (OES). Neither UV radiation nor cytotoxic nitric oxide ($NO^\bullet$) or hydroxyl radicals ($HO^\bullet$), but nitrous oxide (N_2O) and ozone (O_3) were measured. Possible reactions of these gaseous molecules with the aqueous liquid could result in acidification and generation of NO_3^-, NO_2^- and H_2O_2. Furthermore, these species, detected in the gas as well as liquid phases, could serve as reaction partners to generate $NO^\bullet$, $HO^\bullet$, nitrogen dioxide ($NO_2^\bullet$), pernitro acid (ONOOH) and hydroperoxy radicals ($HOO^\bullet$) in the liquid which could be responsible for antimicrobial effects. Concluding, the possible applications of physical plasma in pharmaceutical fields are discussed.

C3-1

COMPREHENSIVE QUALITIY CONTROL OF HEPARINS BY A SIMPLE MICROPLATE ASSAY PROCEDURE

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In 2008, hundreds of serious adverse events demonstrated the health risk by counterfeit heparin and initiated a comprehensive revision of the Pharmacopoia monographs for the quality control of heparin. So far, the proposed purity tests include 1H-NMR-spectroscopy, SAX-HPLC and the Lowry assay, which are sophisticated, expensive and time-consuming, respectively. Here, we present an assay procedure allowing both the simultaneous detection of a wide range of potential heparin falsifications and natural contaminants, whereby this purity testing is directly combined with the determination of the potency of heparin. Samples of pure reference heparin (HR) and heparin spiked (HS) with varying contents of contaminants were examined. The contaminants included falsifications, e.g. OSCS, other heparin imitating sulfated glycans (SG), the FXa inhibitor rivaroxaban (DXI), the thrombin inhibitor argatroban (DTI) as well as the natural contaminant dermatan sulfate (DermS) and BSA as a protein. The assay procedure was performed as follows: A sample of the heparin to be tested (HT) and of a heparin reference (HR) (100 µg/ml) were incubated with 2.5 IU/ml heparinase I for 100 min. Then, the incubated and the non-incubated samples were measured in a novel fluorescence assay using the heparin sensor Polymer-H (FA) and chromogenic anti-FXa- (aXa) and anti-thrombin (aIIa) assays.
The assay procedure provides information on both the purity and the potency of a heparin sample. By the FA, OSCS (LOD 0.5%) and other SG as well as proteins (LOD 0.1 %) are detected in incubated HS by increased fluorescence intensity compared to HR. By the potency assays aXa- and the aIIa-assay, the aXa-and aIIa-activities (aXa-IU/mg and aIIa-IU/mg) of the non-incubated HS (IU/mg) are determined. Remaining aXa- or aIIa-activity of the incubated HS indicates again contamination with any SG (LOD 0.3 %), but additionally with any DXI or DTI (e.g. LOD 0.07 %), resp.. Finally, by using heparin cofactor II instead of antithrombin as reagent in the aIIa-assay, the content of DermS is quantified (LOD 1.0 %). In conclusion, the combination of the classical potency assays, i.e. aXa- and aIIa-assay, with an enzymatic degradation step and a fluorescence measurement using the sensor Polymer-H allows a rapid, simple and comprehensive examination of the quality of heparin just requiring a microplate reader.

C3-2

A METABOLOMICS VIEW ON STAPHYLOCOCCUS AUREUS

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Staphylococcus aureus is a versatile pathogenic bacterium responsible for a wide range of nosocomial infections found in humans and animals. As a commensally microorganism *S. aureus* is resting on mucosa and skin. Most severe forms of staphylococcal infections are endocarditis, osteomyelitis, sepsis and forms of the toxic shock syndrome. Many *S. aureus* strains are able to express a large number of virulence factors like cell-surface exposed proteins, enzymes and toxins supporting invasion into tissues and cells. For survival within the host, several regulatory strategies, defined structural and functional features of virulence factors and the interaction with the core cell metabolism are responsible. These interactions between the eukaryotic and bacterial cells caused e.g. by nutritional limitation, anaerobic life or antibiotic stresses result in an adaption of the microbial virulence factor expression and metabolism to survive within the host environment. There is also an urgent need for new antimicrobial drugs especially against S. *aureus* and its Methicillin and Vancomycin resistant strains (MRSA & VRSA). To find new antibiotic targets or to evaluate the connection between virulence and metabolism in *S. aureus*, we have to understand the physiology of this versatile pathogen and it is therefore of crucial importance to decipher its metabolome. Approaches to understand the metabolic adaption of *S. aureus* towards environmental stresses represent a main focus of our research. In combination with proteomics, the metabolomics approach allows a global view and a better understanding of regulatory systems, dynamic ranges and the control of metabolic pathways of pathogens like *Staphylococcus aureus*. The talk will give an introduction into the life of *S. aureus* and presents recent results of the investigation of its metabolism.

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C3-3

NEW INSIGHTS WITH 'OLD' METHODS? HIGH PRECISION POLARIMETRY AND REFRACTOMETRY: FROM KAISER'S GELATINE TO DETECTING BIOLOGICAL WARFARE AGENTS.

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Determining refractive indices and optical rotation are well-established in characterising substances. These properties are macroscopic and conveniently measurable with optical methods [1]. Current pharmaceutical research, to the contrary, is faced with questions on the intricately accessible nano-scale. However, several nano-effects manifest themselves in easily measurable optical properties. It is therefore interesting to see how high precision refractometry and polarimetry can contribute to current pharmaceutical research, also in combination with other methods: Polarimetric studies have been carried out to shed light onto phase transitions in gelatine (e.g. sol-gel transitions) [2]. In order to ensure effective drug delivery, the interaction of gelatine with agent substances has been studied e.g. for amphiphilic substances and diclofenac [3]. In the class of agent nano-particles, the measurement of size-distributions by laser diffractometry has been shown to be highly sensitive to the refractive index as an input parameter [4], thus emphasising the urgent need for highly accurate refractometry in order to obtain reliable results. Polarimetric techniques are frequently employed in the context of measuring enantiomeric excess in agent synthesis. Furthermore, polarisation-sensitive HPLC has been utilised to detect optically active samples e.g. concentrations of a nerve agent in blood samples [5]. Optical measurements are fast, non-destructive, and, in case of polarimetry, selective for optically active substances. New generation refractometers and polarimeters ensure high accuracy and reproducibility, ease of use and stability in order to deliver a valuable contribution to pharmaceutical research.

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C3-4

INTERACTION STUDIES BETWEEN PEPTIDES DERIVED FROM PHOTORECEPTOR GUANYLYL CYCLASE AND GCAP-2

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We conducted interaction studies between peptides derived from photoreceptor guanylyl cyclase (ROS-GC) and the guanylyl cyclase-activating protein 2 (GCAP-2) using a combination of chemical cross-linking and high-resolution mass spectrometry. ROS-GC is a membrane protein, which increases the concentration of cGMP and regulates the adaptation of the retina in response to light. The activity of the enzyme is Ca^{2+}-dependently regulated by GCAP-1, GCAP-2, and ATP. GCAP is an *N*-terminally myristoylated Ca^{2+}-binding protein containing four EF-hand motifs. It is known that a malfunctioning ROS-GC/GCAP interaction may lead to degenerative retinopathies underlining the importance to understand these interactions in detail. Cross-linking reactions between ROS-GC peptides, that represent potential GCAP binding sites (amino acids 503-522 (GC-peptide 1) and 965-981 (GC-peptide 2) of the full-length protein), and GCAP-2 were performed with and without Ca^{2+} using the homobifunctional amine-reactive, isotope-labeled (D_0 and D_4) cross-linker bis(sulfosuccinimidyl)glutarate (BS^2G). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was used to analyze the intact cross-linked complexes. Cross-linking reaction mixtures were separated by one-dimensional gel electrophoresis (SDS-PAGE). For a detailed structure analysis of the complexes, gel bands of interest were excised and digested with trypsin and Glu-C. The peptide mixtures were analyzed by nano-HPLC/MALDI-TOF/TOF-MS and nano-HPLC/nano-electrospray ionization (ESI)-linear ion trap (LTQ)-Orbitrap-MS. A number of intramolecular cross-links and several cross-linker-modified lysines were identified in GCAP-2. Intriguingly, several cross-links pointed to an interaction between the *N*-terminus of GC peptide 2 and different lysines of GCAP-2. In the excised gel bands, GC-peptides 1 and 2 were identified confirming the proposed interaction between both GC-peptides and GCAP-2. Based on the cross-links between the GC-peptides and GCAP-2, we are currently creating models of the GCAP-2/GC-peptide complexes. Preliminary docking experiments gave a first hint on the orientation of GC-peptide 2 in the GCAP-2/peptide complex. The final aim of this study is to create a structural model of the functional complex between ROS-GC and GCAP-2 in the membrane.

C4-1

CISPLATIN-CONTAINING LIPOSOMES TO INVESTIGATE THE MECHANISMS OF CHEMORESISTANCE IN TUMOUR CELLS

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Cisplatin is one of the most widely used drugs in therapy of advanced ovarian cancer. Resistance of tumour cells against anticancer chemotherapy is a major problem limiting its therapeutic potential. The most frequently observed mechanisms leads to a reduction of intracellular platinum levels, and a diminished cytotoxicity.
Liposomal cellular entry via endocytosis appears a promising approach to circumvent accumulation defects in resistant cells. In this study the potential of cisplatin liposomes to overcome chemoresistance was investigated. A2780 cisplatin-sensitive and -resistant ovarian cancer cells were incubated with holotransferrin-targeted cisplatin-containing liposomes. Cytotoxicity (MTT and ATP assays) and cellular platinum accumulation (flameless AAS) were compared to those of the free drug. For better insights in intracellular processing of liposomal vs. free cisplatin, confocal laser scanning microscopy was applied.
In comparison to the free drug, the liposomal uptake of cisplatin is increased in the resistant cells and decreased in the sensitive cells. Interestingly, the uptake of liposomal cisplatin was nearly identical in both cell lines. The platinum accumulation in both cell lines was correlated with cytotoxicity. Liposomes displayed a higher cytotoxicity in the resistant cells in comparison to the free drug. The measurements of the intracellular ATP levels suggest a modified intracellular trafficking of liposomal cisplatin after endocytotic uptake. Furthermore, despite of their higher cytotoxicity, liposomes may be restricted in the intracellular drug release [1]. This issue deserves further investigation.
With respect to the established benefit of passive liposome targeting to tumour tissues, these findings suggest liposomes as promising tools to gain further insight into the mechanisms of chemoresistance and to potentially overcome it.

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C4-2

NEW DESIGN CONCEPT FOR THE DEVELOPMENT OF 17β-HSD1 INHIBITORS: PROMISING DRUG CANDIDATES FOR THE TREATMENT OF ESTROGEN DEPENDENT DISEASES

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Estradiol (E2), the most important estrogen in humans, is involved in the initiation and progression of estrogen-dependent diseases like breast cancer and endometriosis. Its intracellular concentration is regulated by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) which catalyzes the reduction of the less active estrone (E1). Because of its expression in the diseased tissues, inhibition of 17β-HSD1 is considered as new promising therapy for the treatment of estrogen-dependent diseases. Although several classes of highly potent steroidal and nonsteroidal inhibitors as well as *in vivo* proof of concept for the indication breast cancer are already described, no 17β-HSD1 inhibitor has entered the clinical development so far. For the design of novel potential inhibitors, a new strategy was applied by considering selected amino acids in three areas within the substrate binding site as potential interacting partners. Besides the catalytic tetrade and the C-terminal region (well known as interacting areas of the natural substrate) a rather hydrophobic subpocket located under the catalytic center was chosen as third area. The applied design concept resulted in a new highly potent and selective class of 17β-HSD1 inhibitors. Its development and structural variations led to interesting structure-activity relationships.The developed inhibitors were further evaluated with regard to their selectivity toward 17β-HSD2. This enzyme catalyzes the oxidation of E2 into E1 and thus represents a biological counterpart of the type 1 enzyme. According to the therapeutic concept, relative binding affinity for estrogen receptors α and β (ERα, ERβ) should be as low as possible to avoid any intrinsic estrogenic effects. In contrast to some other inhibitor classes, the intracellular activity (in T47D cells, a breast cancer cell line expressing 17β-HSD1 and 17β-HSD2) of these inhibitors was evaluated and revealed IC_{50}-values in the low nanomolar range. All data obtained make these inhibitors interesting candidates for further preclinical evaluation.

C4-3

LIGHT-ACTIVATABLE TRANS-DIAZIDO PT(IV): BIOLOGICAL ACTIVITY AND THE INFLUENCE OF AMINO LIGANDS

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Light-sensitive Pt(IV) complexes are a new approach to lower adverse drug reactions, increase the selectivity and therefore enhance the efficacy of platinum-based anticancer treatment. They can be used in the photodynamic therapy for the treatment of localized tumors accessible for irradiation.Here we report on the influence of the amino ligand towards the biological activity of three *trans*-diazido platinum(IV)-complexes.

The complexes *trans, trans, trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ (**1**) and *trans, trans, trans*-$[Pt(N_3)_2(OH)_2(NH_3)(pyridine)]$ (**2**) were first described by Sadler. We have now synthesized the piperidine analogue *trans, trans, trans*-$[Pt(N_3)_2(OH)_2(NH_3)(piperidine)]$ (**3**) and studied its photobiological properties. To analyze the cell growth inhibitory potential, an *in vitro* microtiter method with various human cancer cell lines was used. Least active complex was **1**, while **2** and **3** showed similar antiproliferative activity. No cross resistance to oxoplatin was found for **2** and **3**. DNA is considered to be the main target of platinum-based anticancer drugs, thus we studied the interaction of the light activated complexes **2** and **3** with DNA. The binding to calf thymus DNA was studied by a square wave voltammetry assay. After irradiation of **2** and **3** the binding to DNA of was almost completed by 10 min (cisplatin 50% after 180 min). Importantly, high chloride concentrations (100 mM) inhibited the platination of DNA. The decrease in ethidium bromide (EtBr) DNA intercalation was investigated next. For **2** and **3** a greater decrease in EtBr fluorescence was detected compared to cisplatin, indicating the formation of more bifunctional DNA adducts. Unwinding of closed circular supercoiled pUC19 plasmid DNA was analyzed by an agarose gel mobility shift assay. The levels of interstrand cross-linking by **2**, **3** and cisplatin in linear DNA were measured by using the pUC19; the cross-linking efficiency for both complexes is comparable to cisplatin. In conclusion, the introduction of a more bulky ligand increased the cytotoxic potency compared to an ammine ligand, but there was no difference in the photobiological properties between complexes with a cyclic aliphatic amine (piperidine) and a heterocyclic amine ligand (pyridine).

C4-4

NOVEL FLUORESCENT PROTEIN KINASE INHIBITORS

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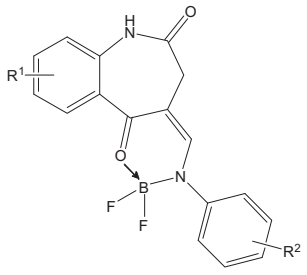

1

Fluorescent tags are feasible structure elements for visualization of the intracellular localization of drug molecules. However, these tags may change both the biological activity and the cellular distribution of the original molecular entity. We here report a novel class of protein kinase inhibitors which shows fluorescence based on the core structure of the molecules and not because of an added fluorescent tag. These novel compounds are (4*Z*)-4-{[*N*-(difluoroboryl)-anilino]methylene}-3,4-dihydro-1*H*-1-benz-azepine-2,5-diones (**1**) which are chemically related to the paullones, a family of inhibitors of glycogen synthase kinase-3 (GSK-3) and of cyclin-dependent kinases (CDKs). Compared to the paullones, **1** shows a modified kinase inhibition profile.

The synthesis, the spectroscopic properties and the chemical stability of **1** will be discussed in the presentation. It will be demonstrated that both the stability and the kinase inhibitory activity of the new compound class is strongly influenced by the nature of the substituent R^2. The inhibition profile in an array of 16 cancer-related protein kinases will be presented as well as the antiproliferative activity for HT-29 human colon carcinoma cells. It was proven by confocal laser scanning microscopy that the novel fluorescent protein kinase inhibitors enter the cytosol, but not the nucleus of HT-29 cells.

C5-1

IMMOBILIZED MONOLITHIC TRYPSIN REACTOR FOR APPLICATION IN PHARMACEUTICS AND PROTEOMICS

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The use of monolithic supports for a wide variety of applications has rapidly expanded during the past few years. The greatest advantages of monoliths comprise their high chromatographic performance even at high flow rates, long life-time of the columns and the possibility of down-scaling, which opens novel applications in the field of microfluidics. Classical reversed phase (RP) or ion-exchange separation media are applied for a broad variety of analytes - small molecules or proteins - while monolithic supports can also be employed for more specialized analytical challenges owing to their ease of modification, which includes almost all known coupling techniques. As such, an affinity enrichment of analytes and separation of enantiomers have been described.
In this work, a monolithic trypsin reactor (MTR) was prepared with the aim of identifying and characterizing proteins. The employed monolithic support was prepared from glycidyl methacrylate, acrylamide, and ethylene glycol dimethacrylate by free radical polymerization and trypsin was coupled to the support using the glutaraldehyde technique. The enzymatic activity of immobilized trypsin was determined using the prototype substrate N_α-benzoyl-L-arginine ethyl ester. Using cytochrome c and bovine serum albumin (BSA) as model proteins digestion parameters, i.e., protein concentration, chaotropic agent, digestion temperature, were optimized. The digests were collected and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF-MS). Moreover, an automated HPLC/MS system allowing an integrated digestion of proteins, separation of the resulting peptides, and a subsequent identification of the poteins by mass spectrometry was established. Finally, the MTR was used for analyzing a protein mixture. Four proteins (310 to 800 ng/mL) were digested in the presence of a 1,000-fold molar excess of BSA. The digest was separated by nano-HPLC and analyzed by MALDI-TOF/TOF-MS/MS resulting in the identification of all four low abundant proteins.
The high efficiency of the MTR combined with a low back pressure and digestion times within a few minutes demonstrate the great potential for high-throughput protein identification and characterization.

C5-2

ACHIRAL–CHIRAL LC/LC–MS/MS COUPLING FOR DETERMINATION OF CHIRAL DISCRIMINATION EFFECTS IN DRUG METABOLISM

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Many physiological processes show a high degree of stereoselectivity, including the metabolism of xenobiotics as catalyzed by cytochrome P450 enzymes. An analysis of these chiral discrimination effects in drug metabolism is essential for an in-depth understanding of metabolic pathways that differ between enantiomers of a given chiral drug or metabolite thereof. Achiral chromatographic separation and structural identification followed by chiral analysis of metabolites from blood specimens usually requires a time-consuming multistage analytical technique.
In an effort to optimize such a complicated analytical scheme, a novel two-dimensional online achiral–chiral liquid chromatography–tandem mass spectrometry (LC/LC–MS/MS) coupling method was developed by using a peak parking technique in combination with a makeup flow system. Metabolites were separated in the first dimension using a C18 reversed-phase system. A makeup eluent of water/methanol (95/5) was split into the flow before storing the metabolites separately on chiral cartridges. Subsequently, the metabolite enantiomers were eluted backward onto the analytical chiral column and separated, and the ratio of enantiomers was determined. The method was successfully validated with respect to limit of detection, linearity, intra- and interday accuracy, and precision. In the course of a human volunteer study investigating the influence of CYP (cytochrome) 2C9 genetic polymorphism on phenprocoumon (PPC) metabolism, we used this new two-dimensional online analytical technique for the analysis of PPC metabolites in plasma. The enantiomeric forms of 4-, 6-, and 7-hydroxy-PPC metabolites as well as two novel metabolites were identified, and the ratio of the enantiomers was calculated. We found that the enantiomeric ratio for the different metabolites in the plasma sample of each measured individual differs markedly from a nearly 100% chiral discrimination for the two new putative metabolites.
This new analytical coupling method possesses general utility in the analysis of chiral discrimination effects, particularly as it relates to pharmacokinetics and dynamics, a scientific field that is rapidly becoming an area of concern and interest.

C5-3

OPTIMIZATION AND APPLICATION OF PEA SEEDLING AMINE OXIDASE MODIFIED BIOSENSORS

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Pea seedling amine oxidase (PSAO) is a Cu-TOPA enzyme catalyzing the oxidative deamination of biogenic amines in the presence of oxygen and water as shown in the following reaction equation [1].

$$R\text{-}CH_2\text{-}NH_2 + O_2 + H_2O \rightarrow R\text{-}CHO + NH_3 + H_2O_2$$

A rapid and simple method for the determination of biogenic amines based on carbon paste biosensors modified with manganese dioxide [2] and PSAO has been developed. This amperometric detector for hydrogen peroxide has been investigated in flow injection analysis (FIA) with an operating potential of +0,4 V vs. Ag/AgCl. Sorensen phosphate buffer 0,1 M; pH 7,5 has been used as media. Immobilization of enzyme has been performed by entrapping DAO in Nafion® films. This method provides good fixation of the enzyme as well as low impact on enzyme activity. The analytical parameters have been investigated and the sensor was used to estimate the biogenic amines content in food samples.

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C5-4

FILLING THE GAP BETWEEN PHARMACOLOGICAL TESTING AND *IN VIVO* FINDING ON THE EXAMPLE OF *BOSWELLIA SERRATA*

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Very often, especially in case of herbal remedies, pharmacological data are not found in line with *in vivo* findings in human. In many cases this may be attributed to the poor bioavailability of the respective pharmacologically active substances *in vitro*. On the example of *Boswellia serrata*, a promising anti-inflammatory alternative that was assigned an orphan drug by the EMA for treating peritumoral edema, it is shown how this gap may be filled.
Until recently, the pharmacological effects of *Boswellia serrata* were mainly attributed to suppression of leukotriene (LT) formation via inhibition of 5-lipoxygenase (5-LO) by 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-β-boswellic acid (AKBA). These two BAs have been also chosen in the monograph of Indian frankincense in the European Pharmacopoiea 6.0 as markers to ensure the quality of the air-dried gum-resin exudate of *B. serrata.*
However KBA and AKBA failed to inhibit LT formation in human whole blood and pharmacokinetic data revealed concentrations of AKBA and KBA in plasma below the pharmacologically effective concentration *in vitro*, putting the hitherto assumed mechanism of action into question. In view of this apparent gap between *in vitro* and *in vivo* data, the Caco-2 model was used to evaluate the contribution of individual boswellic acids to the observed effects. A close look at the clinical indications of *BSE* and the underlying results of clinical trials as well as the experimental data from *in vitro* studies, and all available pharmacokinetic and metabolic data of BAs, resulted finally in a new mechanism of action for *Boswellia serrata*, which is worth to be investigated in further clinical trials and pharmacological studies.

C6-1

ROLE OF THE SECOND EXTRACELLULAR LOOP OF THE ADENOSINE A_{2B} RECEPTOR IN RECEPTOR ACTIVATION

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The human adenosine A_{2B} receptor, which belongs to the familiy of G protein-coupled receptors (GPCRs), plays an important pathophysiological role in inflammatory processes, especially in respiratory diseases, where it appears to have a proinflammatory role. In contrast to the closely related A_{2A} receptor subtype, which mediates antiinflammatory and immunosuppressive effects at low, nanomolar adenosine concentrations, the A_{2B} receptor is typically only activated by much higher, micromolar concentrations of adenosine. The second extracellular loop (ECL2) is known to participate in ligand binding in many GPCRs. The ECL2 of the A_{2B} receptor is several amino acids longer then the ECL2 of the other adenosine receptor subtypes.
In the present study we combine homology modeling, loop simulation and mutagenesis in order to gain deeper insights into the structure and function of the human A_{2B} receptor. The complete ECL2 of the A_{2B} receptor was replaced by the ECL2 of the A_{2A} receptor by overlap extension mutagenesis and selected single amino acid residues were exchanged for alanine by site-directed mutagenesis. The resulting receptor mutants were stably expressed in CHO cells using a retroviral expression system, and characterized by radioligand binding and functional assays. All agonists investigated showed increased efficacy at the loop-exchange mutant as compared to the wildtype A_{2B} receptor, while most single mutants did not show any effect. In contrast to the wild type A_{2B} receptor, the loop-exchange mutant could be activated by the A_{2A}-selective agonist CGS21680 at micromolar concentrations.
The second extracellular loops of the adenosine A_2 receptors appear to play an important role in receptor activation.

C6-2

IN SILICO ANALYSIS OF THE HISTAPRODIFEN INDUCED ACTIVATION PATHWAY OF THE GUINEA-PIG H_1-RECEPTOR

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The histamine H_1-receptor (H_1R) belongs to the rhodopsin-like G protein-coupled receptors. Several studies suggest that the binding of (partial) agonists into the binding pocket of biogenic amine receptors induces a conformational change from the inactive to the active state of the receptors. Meanwhile several crystal structures of inactive and active states of opsin or biogenic amine receptors are known. However, there is only little knowledge about the binding (or unbinding) pathways of ligands into the binding pocket of biogenic amine receptors on molecular level. So far, it was not possible with molecular dynamic simulations to observe the ligand binding and receptor activation. Furthermore, there is nearly nothing known, in which state of ligand binding the receptor gets activated. Thus, the aim was to get more detailed insights into the process of ligand binding and receptor activation. With the recently developed ***LigPath*** algorithm, we scanned the potential energy surface of the binding process of dimeric histaprodifen, a partial agonist at the gpH_1R, into the binding pocket of the gpH_1R, taking also into account the receptor activation [1]. The calculations exhibited large conformational changes of $Trp^{6.48}$ and $Phe^{6.55}$ during ligand binding and receptor activation. Additionally, conformational changes were also observed for $Phe^{6.52}$, $Tyr^{6.51}$ and $Phe^{6.44}$. Conformational changes of $Trp^{6.48}$ and $Phe^{6.52}$ are discussed in literature as rotamer toggle switch in context of receptor activation. Additionally, the systematic scan of the potential energy surface allows to predict favored binding pathways. The calculations indicate that the binding of dimeric histaprodifen, accompanied by receptor activation is energetically preferred. In general, this study gives new insights onto ligand binding and receptor activation on molecular level.

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C6-3

DYNAMIC MOTION INVESTIGATION OF 17β-HSD1 PROVIDES INSIGHTS IN ITS ENZYME KINETICS AND LIGAND BINDING

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Bisubstrate enzymes, such as 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), exist in solution as an ensemble of conformations. 17β-HSD1 catalyzes the last step of the biosynthesis of estradiol and, thus, it is a potentially attractive target for breast cancer treatment. Based on a structural analysis of the available crystal structures, different enzyme conformations were assigned to the putative five steps of the random bi-bi kinetic cycle of 17β-HSD1. Moreover, in order to validate the designed catalytic cycle all-atom molecular dynamic simulations were performed using the four 3D-structures best describing apoform, opened, occluded and closed state of 17β-HSD1 as starting structures. With three of them binary and ternary complexes were built with NADPH and NADPH-estrone, respectively, while two were investigated as apoform. Free energy calculations followed up with the aim to judge more accurately which of the MD complexes describes a specific kinetic step. The analysis of these eight long range MDs revealed an essential role played by backbone and side chain motions, especially of the βFαG'-loop, in cofactor and substrate binding. Thus, a selected-fit mechanism is suggested for 17β-HSD1, where ligand-binding induced concerted motions of the FG-segment and the C-terminal part guide the enzyme along its preferred catalytic pathway.
The elucidation of the kinetic mechanism and of the peculiar role of the flexible βFαG'-loop laid the basis for the identification of a novel binding mode for the bis(hydroxyphenyl)arene derivatives, highly potent inhibitors of 17β-HSD1. In particular, docking studies using an opened enzyme conformer, supported by an exhaustive molecular electrostatic potential investigation, led to the discovery of a novel binding mode for this class of inhibitors. They seem to bind in a synergic manner to the nicotinamide moiety of NADPH via π-π stacking and h-bond formation, hence freezing the enzyme in a „half-switching" state and inducing a dynamic disruption of the enzyme's kinetics. This binding mode was then confirmed by a multiple-trajectory MD approach supported by free binding energy calculations.

Kurzvorträge
Klinische Pharmazie

K1-1

PREVALENCE AND DETERMINANTS FOR THE INTAKE OF INAPPROPRIATE DRUGS IN PRIMARY HEALTH CARE

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Objectives: Drug intake is associated with a risk of drug related problems, e.g. the intake of potentially inappropriate drugs (PIM). The proportion of PIM taken by elderly people in the AGnES-studies (dtsch. **A**rzt-entlastende **G**emeinde-**n**ahe **E**-Health-gestützte **S**ystemische Intervention) was analysed. **Methods:** 744 patients aged >65 years and regular drug intake received a standardized IT-supported, comprehensive home medication review (HMR) conducted by specially qualified AGnES-practice assistants in a community-based, prospective cohort study in the ambulatory health care sector. Out of these patients, 373 received a pharmaceutical intervention by the local pharmacist and a follow-up HMR. The updated Beers'-list was used to detect PIM for patients >65 years as well as drug-condition interactions. GP's diagnoses were extracted from patients' health records. **Results:** 18% (n=134) of the patients received in total n=163 inappropriate drugs during the baseline data collection. Out of these drugs, most prevalent PIM were benzodiazepine derivates (n=45) and non-selective monoamine reuptake inhibitors (n=29). A total of n=25 drug-condition interactions (e.g. Amitriptyline and COPD) were identified. The intake of PIM was associated with self reported falls (phi-value: 0.1074; p=0.0244). Multivariate binary logistic regression showed that the number of taken active substances (OR=1.176; 95%-CI 1.121-1.234, p<0.001) is a determinant for taking at least one PIM. In patients' follow-up data we found an insignificant reduction of the proportion of patients taking PIM from 18.5% (n=69) to 15.3% after pharmaceutical intervention (n=57; p=0.0704; McNemar test). **Conclusions:** In a community based setting a high proportion of patients taking PIM was investigated. Statistical associations with self-reported falls were found. A limited reduction of intake of PIM over the follow up period may have been caused by insufficient knowledge of PIM by pharmacist and GP, respectively. Since study procedures did not focus on PIM, confounding may influence data. A more significant reduction of PIM-intake should be aimed in the future. Further research should employ controlled designs.

K1-2

PROSPECTIVE MULTI-STEP INTERVENTION STUDY TO PREVENT DRUG ADMINISTRATION ERRORS IN PAEDIATRIC SETTINGS

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Introduction Particular pharmaceutical knowledge is required in administering drugs which are often not approved for children. In routine care, drug administration errors are alarmingly frequent in children. Therefore, we conducted a study investigating a monitoring and teaching concept to prevent those errors.
Participants and Methods A multi-step study was approved by the local Ethics Committee and conducted in a general paediatric ward (GPW) and paediatric intensive care unit (PICU). (i) Clinical pharmacists observed the nurses to identify errors in routine drug administration. (ii) An expert panel developed a questionnaire to identify knowledge deficits (KDs) and (iii) presented the answers (1st intervention) followed by (iv) another monitoring. (v) Errors were classified in a decision-matrix for a teaching course (2nd intervention) followed by (vi) another monitoring. Fisher's-Exact-Test and Mann-Whitney-U-Test were used for statistical analysis (significant with p<0.05).
Results The monitoring in GPW assessed 767/1161 processes (66%) with at least one administration error. The error prevalence was 1053/1161 (91%) which decreased to 263/400 (66%, p<0.001) after the 1st and to 254/645 (39%, p<0.001) after the 2nd intervention. Concerning IV drugs in 38/326 processes (12%) the wrong kind of solvent, in 94/326 (29%) the wrong amount of solvent were used. The error prevalence in the wrong kind of solvent decreased to 3/167 (2%, p<0.001; RRR 83%) by the teaching course and in the amount of solvent to 13/87 (15%, p<0.001, RRR 48%) by the questionnaire. The overall prevalence of KDs (response rate 60% vs.64%) was similar in GPW and PICU (20% vs.22%, p>0.05) whereas the prevalence of KDs in subcategories varied between the GPW and the PICU (IV drugs 43% vs.31%, p=0.013, oral drugs 2%vs.19%, p<0.001).
Conclusion Monitoring was an appropriate strategy to identify unexpected high error prevalence in routine drug administration. A multi-step intervention strategy tailored to the prevalence, potential severity, and causes of the errors prevented a high fraction of those identified errors.

K1-3

COMPARISON OF BODY SIZE DESCRIPTORS AS INFLUENTIAL FACTORS IN SIBROTUZUMAB POPULATION PHARMACOKINETICS

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Background: Several monoclonal antibodies including sibrotuzumab have been shown to exhibit body size-dependent pharmacokinetics (PK) [1]. The objective of this analysis was to compare the suitability of different body size descriptors (BSD) as patient specific factors (covariates) in a population PK model, to determine the optimal BSD and to investigate the impact of differences in body size on sibrotuzumab exposure.
Methods: Sibrotuzumab PK has best been described by a two-compartment model with both linear and nonlinear elimination from the central compartment. Body weight (BW) was incorporated as a covariate on 4 structural model parameters [2]. Nonlinear mixed-effect modelling with NONMEM™ was applied to estimate model parameters for the baseline model without BSD covariates and for the models including the following body size descriptors: BW, patient height, body mass index (BMI), body surface area, fat-free mass, ideal body weight, lean-body mass.
Results: (1) Compared to the baseline model, incorporation of body size significantly improved the model performance by reducing unexplained variability. (2) The different BSDs performed similar, with the exception of BMI, being inferior. (3) Covariate relations in patients with median and "extreme" (0.05 and 0.95 percentile) BSD values indicated considerable influence of body size. (4) This influence was confirmed by deterministic simulations of a 12 week treatment period with weekly dosing of 100 mg sibrotuzumab. Minimum Css in patients with "extremely low" body size even exceeded maximum Css of patients with "extremely high" body size.
Conclusion: Body size does have an impact on sibrotuzumab PK. Based on the analyses, no single BSD appears to be superior. Stochastic simulation will be implemented to further evaluate this hypothesis and may ultimately contribute to optimised dosing regimens.
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CARDIOVASCULAR RISK SCREENING AND PREVENTION CARE FOR 50 - 70 YEAR OLD PEOPLE IN COMMUNITY PHARMACIES

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Coronary heart disease is much more frequent in the Northeast of Bavaria, Germany, than in other regions of Bavaria. The aim of this project is, 1st, to evaluate the presence of risk factors for coronary heart disease in this high-risk region and, 2nd, to contribute to cardiovascular risk reduction by preventive Pharmaceutical Care. For this purpose a new screening method is used: People between the age of 50 and 70 years are invited to have their blood pressure, body mass index and other data measured in a community pharmacy. In addition a small blood sample from the finger pad is taken and sent to a specialized laboratory to detect alterations of metabolism. Besides routinely determined parameters as total cholesterol, HDL cholesterol and LDL cholesterol, other lipid values like small dense LDL and VLDL-composition are measured. For people with increased cardiovascular risk Prevention Care Services are offered for twelve months, e.g. Diet Counseling in community pharmacies. Afterwards all participants are tested again.

Thirteen community pharmacies are involved in this project. After 5 months of recruiting (February till June 2010), 1521 participants have already been included. 85 % of them are showing severe cardiovascular risk factors which were mainly caused by unhealthy lifestyle. Therefore cardiovascular risk factors in this region may be reduced by improving lifestyle habits. Within this project community pharmacists offer support to subjects at risk to modify their lifestyle. At the end of the follow-up we will evaluate the effect of the Prevention Care Services on the assessed risk factors for coronary heart disease. If it could be shown, that those pharmaceutical interventions are successful, we aim to expand this project to other community pharmacies.

Kurzvorträge
Pharmakologie & Toxikologie

P1-1

EVIDENCE FOR THE EFFECTIVENESS OF STW5 (IBEROGAST) IN AN EXPERIMENTAL MODEL OF ULCERATIVE COLITIS.

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Our earlier studies with the successful use of STW5 in Trinitrobenzene sulfonic acid –induced colitis as an experimental model of Crohn‘s disease has prompted us to investigate the potential use of the drug in Dextran Sodium Sulfate (DSS) induced colitis as an experimental model reflecting more closely ulcerative colitis in man.
Colitis was induced in rats by feeding them with DSS (5%) in drinking water for one week. STW 5 (Iberogast), a multicomponent herbal preparation of established efficacy in functional dyspepsia and irritable bowel syndrome, was tested in dose levels of 2 ml/kg and 5 ml/kg alongside with sulfasalazine (300 mg/kg) as a reference standard. The drugs were given orally daily for 1 week before initiation of colitis induction and continued during the week of DSS feeding. At the end of the experimental period, animals were sacrificed, the colons examined, and blood samples taken for measurement of relevant parameters.
DSS induced a sharp decrease in body weight which was more effectively normalized by STW 5 than by sulfasalazine. It also led to shortening of colon length and an increase in colon mass index, effects that were reversed by treatment with either drug. Changes in myeloperoxidase, reduced glutathione, glutathione peroxidase, and TNFα induced by DSS were also reversed by STW5 and by sulfasalazine almost to the same extent.
The present findings emphasize the antioxidant and antiinflammatory effects of STW 5 and point to the potential usefulness of STW 5 in the clinical setting of ulcerative colitis.

P1-2

LOCALISATION AND PHARMACOLOGY OF HISTAMINE-INDUCED FREE RADICAL PRODUCTION IN SMALL AND LARGE INTESTINE

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Oxidative stress is a hallmark of the inflammatory reaction and is also involved in many acute and chronic diseases of the intestine. As the generation of oxygen free radicals by intestinal preparations can be stimulated by histamine, we aimed to characterize this effect in different preparations from small and large intestine from brown standard mice (SV1297) and to describe the influence of pharmacological agents, so studying pathogenic mechanisms involved in inflammation and allergy in the gastrointestinal tract.
After excision of the gut, three layers of the wall were prepared and separated into mucosa, tela submucosa and muscularis. The preparations of the gut were studied histologically. Free radical production of the tissue was detected by luminol-enhanced chemiluminescence.
As histology showed, different types of leukocytes are located mainly in mucosal and submucosal layers. Radical production could be stimulated by histamine in a concentration range from 5 to 100 µmol/L, mainly in segments of proximal and distal small intestine, less in colon, and only in the tela submucosa. Neither the intact wall of the gut, nor the pure mucosa or the muscularis could be stimulated by histamine. The effect could be blocked specifically by the H_3-antagonist clobenpropit with an IC_{50} of approx. 0.1 µmol/L; blockers of H_1 and H_2 receptors exerted inhibitory effects only at concentrations more than 10-100fold higher. Similar to the antioxidant trolox, herbal extracts from peppermint and chamomile, as well as STW 5 (Iberogast®), a herbal combination preparation with antiinflammatory properties and containing these extracts, had significant inhibitory effects even in dilutions down to 0.1 µl/ml.
It can be concluded, that submucosal tissue, especially in the small intestine, represents a source of free radicals. This source of free radicals could be important in inflammatory and allergic intestinal diseases, as well as in irritable bowel syndrome, a disease often involving subclinical inflammations, and might be a target for pharmacotherapeutic interventions.

P1-3

ANTI-PROLIFERATIVE EFFECTS OF THE ANTIDYSPEPTIC DRUG STW 5 IN COMPARISON WITH NSAIDS

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STW 5 (Iberogast®) consist of a combination of nine plant extracts and is widely used in the treatment of functional gastrointestinal disorders, including functional dyspepsia and irritable bowel syndrome. Our objective was to determine anti-proliferative effects of STW 5 on colon adenocarcinoma cells (HT-29) in comparison with non steroidal anti-inflammatory drugs (NSAIDs) like aspirin (ASA) or diclofenac (Diclo), which both have been shown to reduce of colon carcinoma risk in clinical and epidemiological trials.
HT-29 cells were treated with Diclo (0.025-0.1 mM), ASA (0.2-2.5 mM), STW 5 (3-300 µg/ml) or its components STW 6 (*Iberis amara* totalis; 12.5 µg/ml), STW 5-K II (peppermint leaves; 50 µg/ml), STW 5-K VII (milk thistle fruit; 50 µg/ml) or STW 5-K VIII (lemon balm leaves; 25 µg/ml). The anti-proliferative effects were measured with Sulforhodamine. Apoptosis was identified by YO-PRO-1® staining. The expressions of the apoptosis-relevant factors p53, Bcl2, BAX , Caspase-3 and the transcription factors subunits of nuclear factor-kappa B (NF-κB)-p65 (RELA), -p50 mRNA were quantified by Real-Time PCR. Treatment with either Diclo (0.1 mM), ASA (2.5 mM), STW 5 (100 µg/ml) or its components STW 6 (12.5 µg/ml), STW 5-K II (50 µg/ml), STW K VII (100 µg/ml) or STW 5-K VIII (25 µg/ml) inhibited proliferation by ca. 50-60 % (ASA or Diclofenac 45-50%) in comparison with untreated cells (control). STW 5 (as well as ASA or Diclo) induced a 3 to 4- fold increase in apoptosis. Moreover, 100 µg/ml STW 5 showed a 20% or 30% induction of Caspase-3 or BAX expression, whereas ASA or Diclo revealed inhibitory effects. Furthermore, 100 µg/ml STW 5 inhibited the Bcl2 and p53 mRNA expression compared to 25 µg/ml. 100 µg/ml STW 5 increased the expression of NF-κB subunits compared with ASA and Diclo. Our data suggest that STW 5 and some of its components show anti-proliferative effects on HT-29 adenocarcinoma cells in vitro, possibly due to an induction of apoptosis cascade via caspase-3 activation, an influence on mitochondrial stability and the activation of the NF-κB pathway. The STW 5 -induced pathway may be different from the pathway initiated by ASA or Diclo.

P1-4

INHIBITION OF PRODRUG ACTIVATION BY HERBAL EXTRACTS AND SECONDARY PLANT METABOLITES

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The bioactivation of pharmacologically inactive prodrugs by drug metabolizing enzymes e.g. esterases, phosphatases or cytochrome P450 (CYP) enzymes is a pivotal prerequisite for their pharmacological action. Examples for prodrugs which are bioactivated by CYP enzymes are irinotecan, cyclophosphamide, desogestrel and the ADP induced platelet aggregation inhibitors ticlopidine and clopidogrel. Recently, the bioactivation of clopidogrel attracted much interest because the concomitant use of clopidogrel and proton-pump inhibitors attenuates the benefit of antiplatelet therapy. In this case it was speculated that the main reason for the impaired clopidogrel bioactivation is the inhibition of CYP2C19 by the proton pump inhibitor omeprazole. Here we describe herbal extracts and secondary plant metabolites which have the potential to interfere with prodrug bioactivation because they inhibit CYP enzymes or esterases. For example, the inhibition of the bioactivation of cyclophosphamide to aldophosphamide was studied using a LC/LC/MS-based screening method. Herbal extracts and natural products were incubated with cyclophosphamide and human liver microsomes or recombinant CYP2B6 and aldophosphamide was quantified with LC/LC/MS in the positive electrospray ionisation mode after derivatisation with *O*-(pentafluorobenzyl)-hydroxylamine. Also, the inhibition of the bioactivation of clopidogrel by herbal extracts was studied using an LC/LC/MS-based CYP2C19 inhibition assay. Since many prodrugs such as irinotecan or methylphenidate are activated by esterases, we also studied the inhibition of esterases in a human liver S9 fraction with p-nitrophenyl acetate as substrate. Among the herbal extracts and natural products with inhibitory activity are peppermint, hawthorne and quercetine, respectively. The obtained data suggest that the bioactivation of prodrugs by CYP enzymes or esterases can be strongly inhibited by herbal extracts or secondary plant metabolites. This implies a risk for drug interactions if natural products with a high inhibitory activity are bioavailable or applied in quantities that exceed the therapeutical relevant doses. Thus, care should be taken if prodrugs are used concomitantly with herbal extracts.

P2-1

HOW DOES THE INSULIN GRANULE BEHAVE BEFORE ITS RELEASE? - TIRF MICROSCOPY ANALYSIS

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Background: The release of a pool of membrane-adjacent secretory granules which are in a primed and docked state and await one final trigger, a depolarization-induced influx of Ca^{2+}, is held responsible for the first phase of glucose-induced insulin secretion. Recently, we observed that 15 mM K^+ led to a 20 mV depolarization and to a lasting increase of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), but only to a modest transient increase in secretion, suggesting that the effect of depolarization on insulin secretion is only incompletely understood.
Methods: Insulin secretion was measured by perifusion of mouse islets and MIN6 pseudoislets and ELISA of the effluate. The $[Ca^{2+}]_i$ of islets and MIN6 cells was measured with the Fura technique. Submembrane granules were visualized by transfection of MIN6 cells with an insulin-EGFP fusion protein and imaging by TIRF microscopy at 37°C. The images were evaluated by a purpose-made program written in MatLab to achieve a complete observer-independent quantitation.
Results: Islets perifused with 5 mM glucose showed only a transient increase in secretion when K^+ was raised to 15 mM. A subsequent elevation to 40 mM K^+ resulted in a prompt overshooting increase in secretion which remained increased as long as 40 mM K^+ was present. In contrast, $[Ca^{2+}]_i$ was continuously elevated by 15 mM K^+ and increased further when K^+ was raised to 40 mM. The same secretion and $[Ca^{2+}]_i$ pattern could be observed with MIN6 pseudoislets. MIN6 cells transfected with insulin-EGFP were therefore used to analyze the behavior of submembrane granules by TIRF microscopy at 5 time points: beginning, prior to high K^+, one each during 15 and 40 mM K^+ and one after washout of high K^+. At each time point a sequence of 100 images was acquired within 12 s. Resident time, number of arriving and departing granules (i.e. return or release), and the total and net distance covered by the submembrane granules were evaluated as mentioned.
Conclusion: 40 mM K^+ affects arrival, departure and residence time of insulin granules, but not movement parallel to the membrane. 15 mM K^+ is moderately effective, which concurs with dynamic secretion measurements. Currently there is no indication that long-term resident granules are preferentially departing during stimulation.

P2-2

TOWARDS FULLY AUTOMATED TIRF MICROSCOPY IMAGE DATA ANALYSIS

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Recently, total internal reflection fluorescence microscopy (TIRF-M) was established as a standard method for observing molecular processes occurring close to the cell membrane. The so obtained image data and sequences of image data (i.e. movies) are usually evaluated with commercial software packages, which determine count statistics of the labeled cell components or tracking of single objects.

The goal of this continuing work is to develop a simple and fast software solution that analyzes the images and movies with special focus on simultaneously tracking all detectable objects in TIRF microscopic images. To guarantee robustness and unbiased analysis, the data analysis shall be almost parameter-free (i.e. ideally needs no expert input).

The current data analysis algorithm comprises the following steps: First, the images and image sequences are preprocessed. Next, the fluorescing cell parts of interest are automatically detected and tracked from picture to picture over time. During tracking, all detected objects are characterized by seven different parameters such as the x- and y-coordinates, ground area, peak height, and the peak integral. The analysis was developed and tested on real-time, live cell imaging TIRF sequences. The results will be presented in the talk.

P2-3

SEX BIAS IN LEUKOTRIENE GENERATION CAUSES GENDER-SPECIFIC EFFICACY OF LEUKOTRIENE SYNTHESIS INHIBITORS

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Leukotrienes (LTs) are powerful lipid mediators of immune and inflammatory responses derived from phospholipase(PL)A_2–released arachidonic acid by the enzyme 5-lipoxygenase (5-LO), aided by the 5-LO-activating protein (FLAP). Prominent diseases (e.g. asthma, allergic rhinitis, rheumatoid arthritis) that are related to LTs are more common in females and gender-disparities in LT biosynthesis exist due to down-regulation by androgens [1]. Here we show that FLAP inhibitors are more efficient in females than in males, and that androgens are responsible for these gender differences *in vitro* and *in vivo*. In human blood from females, FLAP inhibitors (MK886, Bay-X1005 and licofelone) suppressed 5-LO product formation more efficiently than in blood from males, and this difference was abolished by supplementation of female blood with 5α-dihydrotestosterone. Instead, direct 5-LO inhibitors reduced 5-LO product formation in males and females equally well. MK-886 effectively reduced LTB_4 pleural levels in female but not in male rats treated with carrageenan, and MK886 increased survival only of female mice in a model of PAF-induced lethal shock. Administration of testosterone abolished the protective effects of MK886. In view of the current active development of FLAP inhibitors as therapeutics in respiratory and cardiovascular diseases, our data suggests to call for "gender-tailored therapy" in LT-related diseases and prompt for consideration of gender issues in the development and use of drugs modifying LT biosynthesis, in order to optimize medical therapy both for men and women.

References

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P2-4

INFLUENCE OF PREGNANCY ON LEUKOTRIENE FORMATION: A PRIME EXAMPLE FOR PERSONALIZED MEDICINE

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Pregnancy is considered as the most fundamental sex difference and is accompanied by major changes of the immune system which are necessary for a successful outcome of gestation. Pregnancy is characterized by downregulation of cell-mediated immunity and upregulation of humoral immunity as well as of certain components of the innate immune system. Leukotrienes (LT) and other lipoxygenase (LO) products are powerful lipid mediators with major roles in inflammation and innate immunity. Although it is known that the course and characteristics of LT-related diseases (like asthma and allergic rhinitis) change during pregnancy, the regulation of LT biosynthesis has not been investigated under theses conditions. Here, we show that LT synthesis in blood and isolated neutrophils strongly differs during pregnancy from non-pregnant females. Thus, 5-LO product formation in stimulated (Ca-ionophore A23187 or lipopolysacharide/fMLP) whole blood from pregnant donors is significantly higher (1.8-fold) than in blood from female controls. Although increased neutrophil numbers may explain the increased LT formation in blood of pregnants, isolated neutrophils from pregnant donors have significant lower capacities (1.5-fold) for 5-LO product synthesis. Interestingly, plasma from pregnant donors upregulates 5-LO product formation (1.4-fold) in neutrophils from control females. Taking together, complex mechanisms (neutrophilia, upregulating effects of plasma, downregulation of LT synthetic capacity in neutrophils) are involved in regulation of 5-LO product formation during pregnancy resulting in higher LT-biosynthesis in blood from pregnant donors. This higher LT formation in blood from pregnant donors might explain the higher incidence of asthma and allergic rhinitis during pregnancy and represents a prime example for the importance of research in the field of personalized medicine.

P3-1

IMPACT OF EFAVIRENZ ON INTESTINAL AND HEPATIC METABOLISM AND TRANSPORT: INTERACTION STUDY WITH EZETIMIBE IN HEALTHY VOLUNTEERS

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Background: Hypercholesterolemia frequently occurs in HIV-infected patients treated with antiretroviral drugs as efavirenz (EFA). These patients may benefit from combination with the cholesterol absorption inhibitor ezetimibe (EZE). Disposition and lipid-lowering effects of EZE are dependent on intestinal ABCB1, ABCC2 and UGT1A1. These genes are known targets of the nuclear receptors CAR and PXR. EFA was shown to be a potent activator of CAR. In this study, we determined the influence of EFA on disposition and effects of EZE.
Methods: Steady state pharmacokinetics of EZE (10 mg, po) alone and after multiple doses of EFA (400 mg, po) was studied in 12 healthy male subjects. Genome wide expression analysis was performed in duodenal biopsies before and after EFA treatment (8 d). Subsequently, mRNA levels were verified for known CAR targets in intestinal tissue and peripheral blood mononuclear cells (PBMCs). Serum cholesterol and plant sterols were measured to conclude on EZE effects. 4-ßOH-cholesterol and serum bilirubin were determined as surrogates for hepatic CYP3A4 and UGT1A1 activity. *Results:* Intestinal expression of several CAR target genes such as ABCB1, CYP3A4, CYP2B6 and UGT1A1 was not affected by chronic EFA treatment. In well agreement to this, EFA treatment had no effect on the serum levels of EZE but significantly decreased the GLUC exposure (325±152 vs. 466±223ng×h/ml, p=0.005) as caused by inhibition of UGT1A1 (in vitro data). Sterol-lowering effects of EZE were not altered by EFA. On the contrary, serum levels of 4-ßOH-cholesterol were significantly increased (100±33.8 vs. 64.0±24.2 ng/ml, p<0.05). Moreover, chronic treatment with EFA significantly up-regulated ABCB1 (p=0.0007) and CYP2B6 (p=0.0158) in PBMCs of our subjects. *Conclusions:* Chronic treatment with EFA did not influence intestinal genes of metabolism and transport but up-regulated hepatic CYP3A4 as well as ABCB1 and CYP2B6 in PBMCs. The observed influence of EFA on disposition of EZE as obviously caused by modulation of intestinal glucuronidation of EZE seems not to be of clinical relevance.

P3-2

INFLUENCE OF ROUX-EN-Y GASTRIC BYPASS SURGERY ON THE PHARMACOKINETICS OF PARACETAMOL, TALINOLOL AND AMOXICILLIN IN OBESE PATIENTS

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Background: Roux-en-Y gastric bypass, whereby the stomach, the duodenum and the jejunum are circumvented, is an accepted procedure in weight loss surgery. Despite its frequent application and the well documented risk of nutritional deficiencies, only little is known about the pharmacokinetic consequences in these patients. Therefore, this study aims to evaluate the influence of this bariatric surgery on pharmacokinetics of paracetamol (well absorbed along the gut), talinolol (ABCB1 substrate) and amoxicillin (PEPT1 substrate). We hypothesized that absorption of talinolol and amoxicillin is decreased after surgery because of the regional intestinal expression of ABCB1 and PEPT1.
Methods: Disposition of paracetamol (200 mg, po), talinolol (50 mg, po) and amoxicillin (250 mg, po) was studied in a three-period, cross-over study in 8 obese patients (7 females, 1 male, age 22-53 years, body mass index 44.3-61.9) before and after Roux-en-Y gastric bypass surgery. Serum concentrations of paracetamol, talinolol and amoxicillin were quantified using validated HPLC-UV and LC-MS/MS methods. Tissue specimens were taken from the duodenum and the jejunal anastomosis, respectively, before, during and one year after surgery.
Results: Bariatric surgery did not significantly affect C_{max} (2.63±1.07 µg/ml vs. 2.00±0.69 µg/ml), $AUC_{0\text{-}24h}$ (6.11±1.56 µg×h/ml vs. 5.94±3.90 µg×h/ml) and half-life (4.35±2.89 h vs. 3.97±2.47 h) of paracetamol. C_{max} and $AUC_{0\text{-}24h}$ of talinolol (36.5±17.3 ng/ml vs. 42.7±17.2 ng/ml; 350±115 ng×h/ml vs. 403±155 ng×h/ml) and amoxicillin (2.57±1.51 µg/ml vs. 3.84±1.45 µg/ml, p=0.036; 7.47±4.58 µg×h/ml vs. 8.23±1.65 µg×h/ml) tended to be decreased. The differences in disposition were most prominent during the absorption period of talinolol ($AUC_{0\text{-}6h}$, 98.4±23.8 ng×h/ml vs. 142±57 ng×h/ml, p=0.075) and amoxicillin ($AUC_{0\text{-}3h}$, 5.02±2.88 µg×h/ml vs. 6.14±1.66 µg×h/ml).
Conclusions: Roux-en-Y gastric surgery may result in reduced oral absorption of talinolol and amoxicillin by bypassing the respective ABCB1- and PEPT1-mediated absorption windows for both compounds in the proximal intestine.

P3-3

THE SARM-LIKE ACTIVITY OF SUPPLEMENT INGREDIENT NOR-ANDROSTENEDIONE DEPENDS ON ROUTE OF ADMINISTRATION

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19-Norandrostenedione (estr-4-ene-3,17-dione, NOR, structure in fig. 1) represents one of the first prohormones that were marketed as dietary supplements. During metabolism it is converted to the active hormone 19-nortestosterone (nandrolone, 17β-hydroxyestr-4-en-3-one, NT, structure in fig. 1). To characterize its tissue specific androgenic and anabolic potency after s.c. and p.o. administration and to identify potential adverse effects Nor was studied in a hershberger assay. Orchiectomized rats were treated with NOR for 12 days either s.c. (1 mg/kg BW/day) or orally (0.1, 1 and 10 mg/kg BW/day). The tissue weights of the levator ani, the seminal vesicle and the prostate were analyzed to determine the anabolic and androgenic activity. Heart and liver wet weights were examined to identify side effects. Serum concentrations of NOR and its metabolite NT were determined. After s.c. administration NOR stimulates skeletal muscle growth in a highly selective manner but exhibits only weak androgenic activity in rats. In contrast, after oral administration of NOR neither stimulation of the prostate nor the levator ani could be observed in the doses administered in this study.
Interestingly, and in contrast to s.c. treatment, oral administration of NOR resulted in a dose-dependent decrease of body weight. GC-MC analysis revealed that free and glucuronidated NOR and NT were detectable in the serum after oral and s.c. administration and that NOR was converted to NT in comparable amounts independent of the route of administration.
In summary, oral administration of NOR as in “dietary supplements”, at least in the rat, seems to be a very ineffective strategy to increase skeletal muscle mass but seems to be associated with side effects.

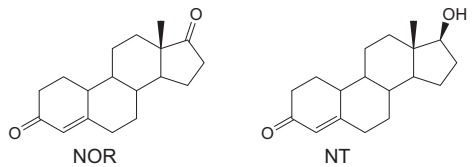

Fig. 1: Chemical structures of Norandrostenedione (NOR) and Nandrolone (NT)

P3-4

COMPARATIVE ANALYSIS OF ESTERASE ACTIVITY IN RECONSTRUCTED HUMAN SKIN MODELS AND EXCISED HUMAN SKIN

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Reconstructed 3D models of human skin and epidermis have mainly been characterized regarding their barrier function and validated for the use in *in vitro* tests for skin corrosion and skin irritation. A great deal less is known about their metabolic capacity concerning activation of prodrugs and toxification or detoxification of chemicals and drugs. In this study, we aimed to compare the esterase activity in commercially available reconstructed human skin and epidermis models and excised human skin. Metabolite profiling of the double ester prednicarbate by HPLC-UV and determination of enzyme kinetic parameters V_{max} and K_M using fluorescein diacetate as a model substrate was performed in parallel. After 24 hours prednicarbate exposure, prednisolone was detected as the main metabolite, however, the relative amount ranked as: full-thickness (epidermis and dermis) skin model ~ epidermis model > excised human skin. The similar results for full-thickness and epidermis models can be explained by the higher esterase activity in human keratinocytes as compared to fibroblasts which contribute very little to the total activity. The formation rates of fluorescein fitted the Michaelis-Menten model. In accordance with prednicarbate metabolism, V_{max} of fluorescein diacetate cleavage was highest in full-thickness skin models and lowest in excised human skin. K_M values did not markedly differ between the test matrices. In conclusion, our results indicate that reconstructed human skin models may be useful to quantitatively address esterase activity of native human skin, although an increased activity compared to normal human skin should be taken into account.

P4-1

THE Gq-COUPLED MUSCARINIC M_3 RECEPTOR GAINS Gi SIGNALING COMPETENCE UNDER CONDITIONS OF ENHANCED cAMP

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Signaling pathway activation was investigated in CHO cells using an optical biosensor technique to measure cellular dynamic mass redistribution (DMR; Epic® system). CHO cells were stably transfected either with the cDNA of the human muscarinic M_2 receptor, preferentially coupling to Gi, the human adrenergic β_2 receptor, preferentially coupling to Gs or the human muscarinic M_3 receptor, preferentially coupling to Gq. Agonists used for receptor stimulation were a dualsteric agonist with M_2-preference and Gi-selectivity (ref. 1), orciprenaline as a β_2 activator and acetylcholine to stimulate the M_3 receptor. To dissect signaling events that underlie the whole cell DMR response, G proteins were targeted with selective toxins. To inhibit Gi, cells were pretreated with pertussis toxin (PTX, 100 ng/ml) overnight. This toxin ADP-ribosylates the undissociated Gαi protein and results in an inactivated heterotrimer. To mask a Gs dependent DMR response, cholera toxin (CTX, 100 ng/ml, overnight) was used. It maximally activates the Gs pathway by ADP-ribosylating the activated α-subunit of Gαs-coupled receptors. As expected DMR signals were eliminated by pertussis toxin in case of hM_2 activation and by cholera toxin in case of $h\beta_2$ activation. In case of hM_3, the signal was not sensitive to PTX but instead augmented after CTX pretreatment. Surprisingly, the M_3 signal of CTX pretreated cells was partially sensitive to pertussis toxin. To check whether an intracellular cAMP elevation may cause the unexpected Gi component, the direct adenylylcyclase stimulant forskolin (10 µM, 2.5 hours) was applied and again the cellular response to acetylcholine included a PTX-sensitive component. These findings suggest that the human muscarinic M_3 receptor is capable of coupling to Gi proteins under conditions of elevated intracellular cAMP. In conclusion the G protein selectivity of the muscarinic M_3 receptor depends on the functional context in CHO cells.

[1] Antony et al. (2009) FASEB J. 23: 442-450. We are grateful to Prof. Dr. Ulrike Holzgrabe (University of Würzburg, Germany) and Prof. Dr. Marco De Amici (University of Milan, Italy) for the design and synthesis of "hybrid 1". We thank Corning Life Sciences for their support on the Epic® system. E.K. and K.M. are supported by the DFG.

P4-2

LINKER LENGTH IS PIVOTAL FOR POTENCY OF DUALSTERIC AGONISTS AT MUSCARINIC M_2 RECEPTORS

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Seven-transmembrane receptors (7TMRs), also known as G protein-coupled receptors (GPCRs), constitute the largest class of cell membrane-bound receptors. Many 7TMRs contain at least one allosteric binding site which is topographically distinct from the orthosteric site recognized by the respective endogenous messenger compound. The muscarinic M_2 acetylcholine receptor is an excellent model to study allosteric/orthosteric interactions as the core region of the allosteric binding site is well characterised. Recently, bisquaternary allosteric/orthosteric hybrid compounds were designed consisting of an allosteric antagonist fragment (from either naphmethonium or W84) linked *via* an aliphatic hexamethylene middle chain with an orthosteric high affinity agonist. Most interestingly, these dualsteric agonists showed subtype-selectivity for the M_2 receptor and biased agonism by exclusively activating the G_i pathway [1]. However, the potency of these hybrids was considerably lower than that of the orthosteric building block.
Here we investigated the role of middle-chain length in affecting the potency of dualsteric agonists. In addition, the allosteric and the orthosteric building blocks were systematically varied. M_2 receptor-mediated G protein activation was measured by [^{35}S]GTPγS binding in membranes of Flp-In™ CHO cells stably expressing the human muscarinic M_2 receptor. Summing up, the findings show that middle-chain elongation considerably increases the potency of dualsteric agonists.

[1] Antony J *et al*. (2009). Dualsteric GPCR targeting: a novel route to binding and signaling pathway selectivity. *FASEB J* **23**: 442-450

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P4-3

THE MULTI-TARGETED KINASE INHIBITOR SORAFENIB INHIBITS HUMAN CYTOMEGALOVIRUS REPLICATION

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Human cytomegalovirus (HCMV) is a major pathogen in immuno-compromised individuals. Here, non-toxic concentrations of the anti-cancer kinase inhibitor sorafenib were shown to inhibit replication of different HCMV strains (including a ganciclovir-resistant strain) in different cell types. In contrast to established anti-HCMV drugs, sorafenib inhibited HCMV major immediate early promoter activity and HCMV immediate early antigen (IEA) expression. Sorafenib is known to inhibit Raf. Comparison of sorafenib with the MEK inhibitor U0126 suggested that sorafenib inhibits HCMV IEA expression through inhibition of Raf but independently of signalling through the Raf downstream kinase MEK 1/2. In concordance, siRNA-mediated depletion of Raf but not of MEK reduced IEA expression. In conclusion, sorafenib diminished HCMV replication in clinically relevant concentrations and inhibited HCMV IEA expression, a pathophysiologically relevant event that is not affected by established anti-HCMV drugs. Moreover, we demonstrated for the first time that Raf activation is involved in HCMV IEA expression.

P5-1

STUDIES ON THE MECHANISM OF ANTIHYPERTENSIVE ACTION OF SOLANUM INDICUM SSP. DISTICHUM.

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Oral treatment of L-NAME hypertensive rats with S. distichum (30mg/kg) for one week after induction of hypertension showed a significant reduction in the elevated SBP and resulted in an
increased diuretic activity manifested by increased renal excretion of water and electrolytes (Na+ and K+), raised NO levels in the sera of treated rats and a reduction of MDA levels in the sera of rats.
The extract further protected against the deleterious effects of hypertension on the kidneys, heart and vascular smooth muscle.
The results obtained confirmed that S. distichum extract has a definite antihypertensive activity in this model. This antihypertensive effect could be due to a variety of factors acting simultaneously, namely, a) preservation of NO vasorelaxant properties by increasing its level in sera of hypertensive rats, b)maintenance of the cellular antioxidant capacity, c) enhancement of pressure natriuresis and d) protection against hypertension-induced organ damage.
The ethyl acetate and methanol (50%) fractions of the extract showed highest activity, indicating that polyphenolic compounds, which are highly soluble in the ethyl acetate and methanol, may have been responsible for the antihypertensive activity of the extract.

P5-2

ANALYSIS OF REQUIREMENTS FOR DIMERIZATION OF NITIRIC OXIDE SENSITIVE GUANYLYL CYCLASE

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Nitric oxide sensitive guanylyl cyclase is the physiological receptor for nitric oxide (NO) and nitric oxide releasing drugs. Its second messenger cyclic GMP is crucial for vasodilatation, penile erection, platelet disaggregation and neurotransmission. The heterodimeric enzyme is formed by a β_1-subunit and either an α_1- or an α_2-subunit. Dimerization of the enzyme is a prerequisite for its catalytic activity. There is conflicting evidence which parts of the subunits are mandatory for heterodimerization. Wagner et al. (J Biol Chem 2005; 280:17687-17693) show that amino acids 61-128 of the α_1 subunit are mandatory for quantitative heterodimerization.

In the current study we purified the β_1-subunit after co-expressing different α_1 deletion mutants using an analogous strategy to the study of Wagner et al. 2005. We found preserved quantitative dimerization with β_1 despite truncation of 259 or 364 amino acids of the α_1-subunit. Analogous amino-terminal truncation of the β_1 subunit ($\beta_1\Delta N_{H\text{-}NOX}$) also led to preserved heterodimerization. In addition we used fluorescence resonance energy transfer (FRET) based on fluorescence lifetime imaging using fusion proteins of the β_1 subunit with EYFP and the α_1-subunit and its deletion mutants with ECFP. Analysis of the respective combinations in HEK-293 cells showed that the fluorescence lifetime was significantly shorter for α_1/β_1, $\alpha_1\Delta N_{258}/\beta_1$ and $\alpha_1\Delta N_{363}/\beta_1$ than the negative control. We conclude that the amino-termini of the α_1- and the β_1-subunit are dispensable for quantitative dimerization.

Next we asked whether dimerization depends on chaperone systems or cellular organelles. Using a cell free expression system based on eukaryotic Sf9 cells (EasyXpress®, Qiagen), we successfully co-expressed the α_1 and β_1 subunits as detected by Western blot analysis. In contrast to co-expression in intact Sf9 cells, no catalytic activity could be detected in the cell free Sf9-system. This indicates that chaperones systems or cellular organelles present in intact Sf9 cells, but absent in the cell free Sf9-system are vital for formation of an intact heterodimeric enzyme.

P5-3

CYCLIN DEPENDENT KINASE 5 (CDK5) REGULATES ENDOTHELIAL CELL MIGRATION AND ANGIOGENESIS

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Angiogenesis contributes to various pathological conditions including arthritis, psoriasis, diabetic retinopathy, macula degeneration, and cancer. During recent years, the search for anti-angiogenic compounds and their molecular targets has been intensified, focusing on the inhibition of vascular endothelial growth factor (VEGF) signalling. Due to the resistance against existing anti-angiogenic therapy an urgent need exists to understand the molecular basis of vessel growth and to identify new targets for anti-angiogenic therapy.
Here we show that Cdk5, an important modulator of neuronal processes, regulates endothelial cell migration and angiogenesis, suggesting Cdk5 as a novel target for anti-angiogenic therapy. Inhibition or knockdown of Cdk5 reduce endothelial cell motility and block angiogenesis *in vitro* and *in vivo*. We elucidate a specific signalling of endothelial Cdk5. In contrast to neuronal cells, the motile defects upon inhibition of Cdk5 are not caused by an impaired function of focal adhesions or microtubules, but by a modulation of the actin cytoskeleton. In the endothelium, Cdk5 regulates the formation of lamellipodia, actin-based protrusions at the leading edge of migrating cells that represent a prerequisite for cell motility. The effect of Cdk5 seems to be mediated by the small GTPase Rac1. Inhibition of Cdk5 decreases the activity of Rac1 at the leading edge, indicating Rac1 as a novel downstream effector of Cdk5.
Our work elucidates Cdk5 as a crucial new regulator of endothelial cell migration. It suggests Cdk5 as a novel, pharmacologically accessible target for anti-angiogenic therapy and provides the basis for a new therapeutic application of Cdk5 inhibitors as anti-angiogenic agents.

P5-4

FLAVOPIRIDOL PROTECTS AGAINST INFLAMMATION BY INHIBITION OF CDK9

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Flavopiridol (FP), a natural compound-derived pan-CDK inhibitor, is used in clinical studies for the treatment of different types of malignancies. We hypothesized that FP possesses anti-inflammatory properties. Hence, we investigated the impact of FP on inflammatory processes *in vivo* and *in vitro* and studied the underlying mechanisms of action in detail.
In vivo, FP effectively protected against concanavalin A-induced inflammatory liver injury in mice as it inhibited the rise in serum levels of transaminases (AST, ALT), reduced necrosis, and lowered infiltration of neutrophils. Studying leukocyte-endothelial cell interactions *in vivo* in venules of the mouse cremaster muscle, we found that FP reduces the TNFα-evoked leukocyte adherence and transmigration. Neutrophil adhesion to endothelial cells was also reduced *in vitro*. This is due to an inhibition of endothelial cell adhesion molecule (E-selectin, ICAM-1) protein and mRNA expression, both *in vitro* and *in vivo*. Mechanistically, FP decreased TNFα-induced NF-κB reporter gene expression, but did not influence NF-κB DNA-binding activity, p65 translocation, degradation of IκBα, and IKK activity. In order to elucidate the basis of these surprising results, we performed a cellular kinome profiling (PepChip microarray). FP inhibited the kinases LIMK1, JNK1, CK2, and PKC-θ. However, by gene silencing or pharmacological inhibition of these kinases, we found that none of them is significantly involved in ICAM-1 regulation. We then tested FP for its inhibitory profile in a recombinant kinase panel: FP (≤100 nM) inhibited CDK4, 6, 8, and 9 more potently than other (cell cycle-related) CDKs. By using shRNA and a specific inhibitor, we identified CDK9 as the kinase responsible for the effect on ICAM-1.
In summary, we revealed FP as a potent anti-inflammatory compound *in vivo*. FP effectively blocked the interaction of leukocytes and endothelial cells by inhibition of endothelial cell adhesion molecule expression. Mechanistically, FP potently reduced NF-κB-driven transcription, but does not interfere with NF-κB activation. This effect is related to the inhibition of the transcription-associated kinase CDK9.

Poster
Pharmazeutische Technologie

T001

REDUCTIVELY DEGRADABLE LINEAR POLY(ETHYLENE GLYCOL)-POLY(ETHYLENE IMINE)–COPOLYMERS FOR THE DELIVERY OF NUCLEIC ACIDS

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The delivery of nucleic acids with polymers has become a promising tool for the modulation of gene expression inside cells. Branched poly(ethylene imine) (bPEI) is still the gold standard, but also shows a significant toxicity to cells. In order to overcome this problem derivatives of this polymer were proposed, especially block copolymers from linear PEIs of low molecular weight and poly(ethylene glycol) (PEG). While all other approaches created branched, graft copolymers, we propose strictly linear, redox-sensitive PEG-PEI block copolymers. We hypothesize that this strategy is more favourable for the formation of well-defined and better shielded nanocarriers because the sterically isolated PEG does not interfere with the interaction between PEI and the nucleic acid. We are sure that the polyplexes (complexes of nucleic acids and polymers) obtained from these copolymers will also show excellent stability and good diffusion in extracellular matrices. Furthermore, these polyplexes are reductively degradable inside cells which will decrease their cytotoxicity. To proof our hypothesis we synthesized redox-sensitive, linear PEG-PEI copolymers and tested their complexation ability of nucleic acids. Additionally, we investigated their diffusion in the extracellular matrix of a 3D-tumour model.

T002

DRUG DELIVERY SYSTEMS BASED ON MODIFIED HYDROXYETHYL STARCH

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Protein - based pharmaceuticals are of increasing importance for the treatment of various diseases like cancer or inflammatory disorders. Since such therapeutic proteins often show fast degradation in the human body, a protecting carrier system that releases the protein in a controlled way is needed to maintain the therapeutic concentration over a certain period of time. Hydrogel microspheres seem to be a promising drug delivery system fulfilling this function. Previously described microspheres based on hydroxyethyl starch (HES) modified with hydroxyethyl methacrylate (HEMA) showed an undesired strong initial burst release and insufficient polymer solubility. Therefore, polyethylene glycol methacrylate ($P(EG)_nMA$) was used as new crosslinkable substituent to solve these problems. The hydrophilicity can be tailored by simple variation of reagent ratios, reaction time and length of the polyethylene glycol spacer. The modified HES can be crosslinked within 15 min. by photopolymerisation using the photoinitiator Irgacure® 2959 at 366 nm (3.5 mW/cm^2). The resulting transparent hydrogels were characterized by swelling measurements in phosphate buffer (pH 7.4) and oscillation rheology and show good mechanical properties. Storage moduli from 200 - 7500 Pa (stress: 1 - 40 Pa, frequency: 1 - 100 Hz) were achieved and the swelling ratio can be varied in a wide range. By increasing the polymer concentration from 10 wt% to 20 wt% a distinct increase in mechanical strength can be obtained. A variation of the initiator concentration (0.015 - 0.1 wt%) or irradiation time (10 - 30 min) seems to barely influence the gel stability. However, at high initiator concentrations and long irradiation times the resulting hydrogels become more fragile. First degradation studies confirm the enzymatic degradation of the HES- $P(EG)_nMA$ backbone by α-amylase and the long - time swelling experiments show degradation of the polymer network by hydrolysis.
HES- $P(EG)_nMA$ was also used for the preparation of microspheres via a water-in-water emulsion. After purification and lyophilization microspheres with a size of ~ 10 µm were obtained which showed high encapsulation efficiency for FITC-dextran 70 kDa of > 70 %. Release studies with this FITC-dextran demonstrated almost no burst release but a steady release over months.

T003

INFLUENCE OF BIOMATERIALS ON MITOCHONDRIAL FUSION IN VITRO

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Mitochondrial fusion is an event, which occurs continuously in cells to form large networks. It is the result of a cell´s effort to adapt and response to various metabolic and environmental stimuli with the ability to redistribute impaired and not impaired proteins as well as mutated and wild-type mtDNA. This process is highly important to maintain mitochondrial function.
Mitochondrial fragmentation on the other hand can be the cause or consequence of mitochondrial dysfunction. The enhancement of mitochondrial fusion could therefore be an important tool to normalize the distribution of mutated and wild-type mtDNA and is hence a desired therapeutic approach in the field of mitochondrial medicine.
Our overall goal was to explore if we can make use of mitochondrial drug targeting strategies and enhance mitochondrial fusion with the help of bio- or nanomaterials. In this study we investigated the influence of biomaterials on mitochondrial fusion in vitro using a PEG-derivative and isolated mitochondria from mammalian cells which were either transfected with a green or red fluorescent protein (GFP or RFP).
Mitochondrial fusion of green and red fluorescent mitochondria was analyzed by flow cytometry as well as confocal microscopy. Fusion efficiency was quantified by the flow cytometry results.
The analysis revealed a higher percentage of green and red fluorescent double positive events, that means fused mitochondria, in PEG-treated samples compared to a usual fusion strategy of mitochondria in vitro.
We thus could show that it is possible to enhance mitochondrial fusion in vitro using biomaterials, such as PEG-derivatives which can be a promising step towards the enhancement of mitochondrial fusion in cells as well.

T004

PLASMA VOLUME EXPANDERS AS POTENTIAL DRUG DELIVERY SYSTEMS – AN *IN VIVO* STUDY UTILISING NONINVASIVE NEAR INFRARED FLUORESCENCE OPTICAL IMAGING

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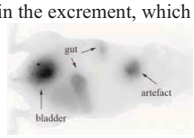
Hydroxyethyl starches (HES) and Dextrans (DEX) are biodegradable polymers that are commonly used as plasma volume expanders (PVE). As they are biocompatible and nontoxic they could be used as drug delivery systems with prolonged circulation time. Three different PVE's were used in this study: HES 200K, HES 450K and Dextran 500K. Aim of this study was to investigate the body fate of these polymers. For that purpose noninvasive near infrared fluorescence optical imaging was utilised.
The polymers have been modified to introduce an amine function that enables a stable amid linkage with carboxyl function carrying fluorescent molecules. Substitution degree was determined utilising H^1-NMR (Gemini 2000, 400 MHz). The polymers were labelled with an IR fluorescent dye (800CW from LI-COR). *In vivo* imaging studies were performed with a fluorescence imaging system (Maestro, Cambridge Research & Instrumentation) in nude mice (SKH1) from Charles River Lab (n=4). 50 µl isotonic solution of the polymers were intravenously injected into the tail vein of nude mice. The experiments with HES 450K and DEX 500K are still running and will be presented on the poster.
For HES 200K the IR fluorescent signal was detectable in the total body. Immediately after injection an accumulation of HES could be found in the bladder, indicating that a fraction of HES molecules has a molecular weight below renal excretion barrier. The bladder signal became weaker after 5 hours due to emiction but was still predominant after 24 hours compared to the total body signal. The urine was intensively fluorescent even after 24 hours. After 150 minutes an artefact in the area of the gallbladder and a large fluorescence signal in the intestinal loops could be observed indicating hepatic clearance (figure). HES could be also found in the excrement, which was collected during 24 hours. The distribution of HES in mouse body could be observed for 72 hours. We expect even longer circulation for HES 450 and DEX 500.



Left: Distribution of HES 200 in a nude mouse after 330 minutes (colour inverted). High concentrations are dark.

T005

A NEW VISIBLE-LIGHT PHOTOINITIATING SYSTEM FOR BIOMEDICAL APPLICATIONS: SYNTHESIS AND CHARACTERIZATION

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This study presents the synthesis and characterization of a new visible-light photoinitiating system for crosslinking of hydroxyethyl starch modified with hydroxyethyl methacrylate (HES-HEMA). This polymer can be crosslinked and can give biocompatible and biodegradable hydrogels which are suitable as drug delivery systems.
Light-activated dental composites are widely used in clinical restorative dentistry. Photopolymerization in this application is commonly carried out using an initiator system comprising an α-diketone, such as camphorquinone (CQ), together with an amine reducing agent (coinitiator). For preparation of hydrogels by photocross-linking CQ is disadvantageous because of its poor solubility in water. Additionally, biocompatibility and toxicity of the coinitiators and its solvents are potential concerns. Thus attempts have been made to replace the simple amine coinitiators as e.g. dimethylaminoethyl methacrylate by less toxic alternatives. For example N-phenylglycine and L-arginine have been used as alternative new coinitiators, which are less toxic.
To overcome the solubility problems carboxylated camphorquinone CQ-COOH was synthesized as a new water soluble photosensitizer. The chemical structure of CQ-COOH has been proven using different characterization methods e.g. NMR, MS, FT-IR, and UV-VIS spectroscopy. CQ-COOH is completely soluble in water and absorbs visible light between 400-490 nm with an absorption maximum at λ_{max} = 457 nm.
In combination with amine coinitiators such as L-arginine CQ-COOH shows a higher photo-reactivity than CQ for photocrosslinking of HES-HEMA. The resulting hydrogels show better mechanical properties and smaller mesh sizes in the network. Cytotoxicity testing has been conducted for all ingredients of the new photoinitiating system by investigating cell viability by MTT-assays. It can be noted that, both CQ-COOH as photosensitizer and L-arginine as coinitiator alone only show minor cytotoxicity towards human gingival fibroblasts cells (HGFib).

T006

DEVELOPMENT OF OPHTHALMIC FORMULATIONS FOR POORLY WATER-SOLUBLE DRUGS: USING POLYETHYLENGLYCOLES

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Drugs like Cyclosporine A or a number of corticoids are known to be efficient tools in the therapy of prevalent ophthalmic diseases like the keratokonjunctivitis sicca. They could help to move from a palliative to a causal therapy. Unfortunately, these potent drugs suffer from low solubility in water and can, therefore, not be administered via aqueous solutions. Oily eye drops would be an alternative, however, their administration leads to irritations on the eye surface.
Liquid polyethylenglycoles (PEGs) would be potent solvents since they are non-aqueous, unlimited water miscible and combine qualities of hydrophilic and lipophilic solvents.
Therefore, we hypothesized that it would be possible to create in situ precipitating systems for Cyclosporine A with PEGs as solvents. Upon contact of the PEG formulation with the tear fluid the rapid increase of the water content induces a precipitation of the drug.
For our investigations we selected two different types of PEG, a polyethylenglycol-monomethylether and a polyethylenglycoldimethylether, both with an average molecular mass of 500 Da. To have further influence on the precipitation, with respect to the point of precipitation (PoP) and the resulting particle sizes, we used Solutol® HS15 (BASF), a Macrogol-15-hydroxystearate as an amphiphilic additive.
Using high performance liquid chromatography we observed an outstanding solubility of Cyclosporine A in both PEGs. With a high throughput method for turbidimetric analysis of drug precipitation, we showed that with decreasing concentrations of drug the amounts of water which are necessary to induce a precipitation, increased. In the presence of Solutol® these levels could be increased further.
Using dynamic light scattering and light microscopy we could show that particle size and particle size distributions at the PoP decreased and were generally more stable with increasing levels of Solutol®.
We could thus show that PEGs can be effective carriers for poorly water-soluble drugs and a promising approach for their ophthalmic application.

T007

PREPARATION OF SMALL HYDROGEL MICROPARTICLES AS ACCEPTOR COMPARTMENTS FOR DRUG TRANSFER STUDIES

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Lipid nanoparticles are being investigated as intravenous drug delivery systems, in particular for lipophilic drugs. It is, however, difficult to analyze the release behavior of such substances from colloidal carriers under realistic conditions. As a new approach to overcome this difficulty, a release setup based on the transfer of the lipophilic drug into a colloidal lipophilic acceptor system incorporated into Ca-alginate microbeads was suggested [1]. The microgel particles were formed by electrostatic droplet generation and hardened in a $CaCl_2$-solution. They had particle sizes of about 330 to 1350 µm, depending on the diameter of the needle applied for droplet generation. Tyloxapol-stabilized trimyristin nanoparticles were incorporated into the hydrogel particles as lipophilic acceptors. The donor system also consisted of these nanoparticles and contained the lipophilic dye Nile red as drug model. In transfer experiments with water-diluted, nanoparticle-containing acceptor microbeads and a donor-acceptor lipid mass ratio of 1 + 25 dye transfer was not completed within one hour regardless of microbead size. Presumably, this slow transfer was caused by the relatively large size of the microbeads.
In the present study, smaller microgel particles were prepared to verify this assumption and to obtain a lower diffusion barrier as an approach to create more *in vivo*-like release conditions. Smaller alginate particles could be prepared by spraying a mixture of lipid nanoparticle dispersion and low viscosity Na-alginate (1 %) into a $CaCl_2$-solution (5 % w/w) using the two-fluid spray nozzle (diameter: 0.7 mm) of a BÜCHI Mini Spray Dryer B-191. After hardening, the microbeads were washed with ultrapure water and filtered off via a paper filter. A median particle size of about 45 µm (d_{10} = 15 µm, d_{90} = 110 µm) was determined by laser diffraction. Transfer studies with these small particles revealed a considerably faster transfer of Nile red than with larger microbeads. It was already completed after a few minutes.
In conclusion, smaller Ca-alginate microbeads lead to a very fast transfer. The transfer system with the small microbeads is now closer to *in vivo*-like release conditions and is an interesting approach to investigate the transfer of lipophilic drugs also to other lipophilic systems.

[1] Strasdat, B., Bunjes, H., Poster presented at the DPhG annual meeting, Jena, 2009

T008

RELEASE PROPERTIES OF HYDROGEL DRUG CARRIER SYSTEMS CHARACTERIZED BY MAGNETORELAXOMETRY

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Hydrogels and hydrogel microparticles are under investigation as new delivery systems for bioactive molecules, e.g. proteins. With their ability to incorporate a huge amount of water or buffer, they offer optimal conditions for a long *in vivo* stability of proteins. The release of proteins occurs by diffusion and hydrogel degradation. In this study, we used polymers based on hydroxyethyl starch (HES) modified either with hydroxyethyl methacrylate (HEMA) or polyethylene glycol methacrylate ($P(EG)_6MA$) as cross-linkable substituents for the production of hydrogels and hydrogel microparticles. In order to study the release properties of the HES-HEMA and HES-$P(EG)_6MA$ hydrogels, superparamagnetic nanoparticles (MNP) were incorporated into the gels to mimic biomacromolecules . By analyzing the magnetic relaxation behavior of the MNPs, the fractions of physically entrapped (immobilized) and mobile nanoparticles can be determined. The hydrogels were produced with various UV irradiation times to investigate the optimal cross-linking conditions for the different polymers and the influence on the release profile. Furthermore, MNPs were incorporated into HES-$P(EG)_6MA$ microparticles during the water-in-water emulsion preparation process. Buffered aqueous polyethylene glycol (MW = 12,000) and HES-$P(EG)_6MA$ solutions were gently vortexed, the latter containing the photoinitiator and the magnetic nanoparticle suspension. The resulting emulsion was exposed to UV light for cross-linking of the HES-$P(EG)_6MA$ containing droplets. After multiple washing steps, the microsphere emulsion was used to study the release profile of MNPs emerging from the hydrogel microspheres over several weeks. α-amylase was added to all systems to accelerate the degradation process.
This work was financially supported by the DFG via SFB 578.

T009

SYNTHESIS OF POLY-L-CYSTEINE AND ITS EFFECT ON PARACELLULAR DRUG TRANSPORT ACROSS CORNEAL EPITHELIUM

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The limiting factor for the bioavailability of many hydrophilic drugs is their insufficient paracellular absorption. The absorption barrier is represented by epithelial cell membranes which are interconnected by tight junctions. The formation of tight junctions is supposedly controlled by protein tyrosine kinase which is influenced by the presence of reduced glutathion (GSH). Thiolated polymers, so-called thiomers, show a permeation enhancing effect by affecting the GSH/GSSG equilibrium via their thiol groups and are regarded as a promising approach to improve drug uptake [1]. This study describes the synthesis of poly-L-cysteine (PLC), a homopolymer of L-cysteine, as a novel thiomer. Furthermore, the influence of PLC on corneal cell viability and drug permeability was studied.
PLC was synthesized from Tmob-protected L-cysteine by formation of cyclic cysteine N-carboxyanhydride and subsequent polymerization initiated by n-hexylamine. The product was water-soluble up to a concentration of 30 g/L and its molecular weight ranged between 36-38 kDa as determined by SEC. The amount of free thiol groups determined via Ellman's reagent was 1.7 µmol thiol groups per gram poly-L-cysteine in average.
The influence of PLC on the cell viability was tested using the different in vitro assays CellTiter-Blue® (conversion of resazurin), CytoTox-ONE™ (LDH release) and CellTiter Glo® (ATP presence). PLC showed only a minor or no ill-effect on the survival of corneal epithelial cells (cell viability more than 80%). Permeation studies were performed in vitro using human corneal epithelial cells (HCE-T) grown on a polycarbonate filter forming tight cell layers. Transport experiments were carried out with the model compound sodium fluorescein (SF) as paracellular marker. The application of PLC resulted in a 25fold increase of the SF permeation coefficient ($8.5 \cdot 10^{-6}$ cm/s in the presence of PLC; $3.4 \cdot 10^{-7}$ cm/s in control experiment).

[1] Bernkop-Schnürch et al. (2004) Thiomers: potential excipients for non-invasive peptide delivery systems. Eur J Pharm Biopharm 58:253-63.

T010

CHARACTERIZATION OF POLY(LACTIC-CO-GLYCOLIC ACID) NANOPARTICLES CONTAINING LANTHANIDES

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Poly(lactic-co-glycolic acid) is a copolymer which is used in a host of FDA-approved therapeutic devices, owing to its biocompatibility and biodegradability. Depending on the monomers' ratio used for the polymerization, different forms of PLGA with different properties can be obtained. In this study, Resomer® RG 502H with a ratio of 50:50 lactide to glycolide was used.

A method for preparation of nanoparticles containing different lanthanides by adaptation of a published protocol was developed, based on a solvent-evaporation-method[1]. Structural investigation of these particles was done by dynamic light scattering (DLS), transmission electron microscopy (TEM) and small angle neutron scattering (SANS) to investigate particle size and particle shape. The particles typically depict a size range from 80 up to 500 nm with a mean diameter of 200 nm. Target load in the case of erbium as heavy metal was determined by UV-spectroscopy after complete dissolution of the particles in DMSO.

Biocompatibility and toxicity of the particles was tested in cell culture. A549 cells, a human lung carcinoma cell line, were incubated with different concentrations of drug-containing polymer for different time periods, cell viability was tested afterwards via 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay, adapted from literature[2]. The amount of purple formazan built by reduction of MTT was measured spectro-photometrically by dissolving the dye in DMSO. No specific toxic effects of our particles were found in comparison to empty particles.

Different applications for our system can be foreseen, i.e. imaging purposes and others are likely to follow.

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2. Mosmann, T., *Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays.* J Immunol Methods, 1983. **65**(1-2): p. 55-63.

T011

SOLVENT DISPLACEMENT METHOD FOR THE PREPARATION OF HSA-NANOPARTICLES

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Nanoparticles prepared of human serum albumin (HSA) represent promising carriers for drug delivery. Protein-based particles can be obtained by several methods. The objective of the present study was the establishment of a solvent displacement method for the preparation of HSA-nanoparticles. Therefore the influence of different organic solvents, stirring speed, and needle diameter on particle size and size distribution of the produced nanoparticles was investigated. The experimental approaches focused on the organic solvents ethanol, acetone, and isopropanol in different concentrations and one experimental set with a combination consisting of methanol and ethanol. Further the influence of different stirring speeds between 200 and 1000 rpm and needle diameters between 0.7 mm and 1.8 mm were tested. Size and size distribution measurements were performed by PCS.
Large particles with a broad size distribution were received when using high concentrated ethanol, acetone and isopropanol as organic solvent. After reducing the concentration of the organic solvent, nanoparticles of a decreased size could be achieved. The combination of methanol and ethanol led to the smallest particles of the study with a particle diameter below 50 nm and a monomodal size distribution. The investigation of the needle diameter pointed out that the resulting particles became smaller with increasing diameter, whereas the variation of the stirring speed showed no significant influence on the particle preparation.

T012

CHARACTERIZATION OF NANOSTRUCTURED POLY-ELECTROLYTE PEPTIDE COMPLEXES

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Self assembly is ubiquitous in chemistry, materials science and biology. It is a widely used term that describes the phenomena of self-organization. According to the literature it is well known that polyelectrolyte-surfactant systems self assemble [1]. In our work we investigated the interaction between oppositely charged polyelectrolytes and small peptides and their ability to undergo similar complexation. Different anionic polylectrolytes (sodium hyaluronate, sodium carboxymethylcellulose and xanthan gum) which differ in their chemical composition and their charge density were examined in order to determine their influence on the structural composition of the aggregates. A positively charged decapeptide was used as counterpart. The nature of the polylelectrolyte-peptide complexes was investigated in dilute solutions as well as in solid state.
Cryogenic-temperature transmission electron microscopy (cryo-TEM) revealed that the interaction between the anionic polyelectrolytes and the oppositely charged peptide led to the formation of nanofibers. Our investigations showed that their diameter, length and shape varied with the used polyelectrolyte. The interaction of the peptide with the polyelectrolyte caused a complete change of conformation, which was analyzed by circular dichroism (CD). Light microscopy pictures of the solid state of the complexes provided further morphological information.
In conclusion, we successfully developed nanofibers by the self assembly of polyelectrolyte-peptide complexes. On the one hand the self assembly is based on electrostatic interactions between oppositely charged molecules. On the other hand the hydrophobic interactions between the peptide molecules play an important role in structural organization. By using polyelectrolytes with different properties the length, shape and diameter of the nanofibers can be adjusted. These nanofibers may have a potential application as a controlled release system

[1] Kötz, J. (2001) Prog. Polym. Sci. 26: 1199-1232

T013

COMPARISON OF NANOPARTICULAR IRON FORMULATIONS FOR PARENTERAL USE – ARE THEY SIMILAR AND READILY EXCHANGEABLE?

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Parenteral iron is today widely used for the treatment of iron deficient anaemia. From initial widespread use in nephrology the use has in recent years spread to other disease areas such as gastroenterology, cardiology, oncology, pre/post operatively, obstetrics and gynaecology. Historically, the first parenteral iron preparations were toxic, being administered as an iron oxyhydroxide complex. The problem was circumvented with the introduction of compounds where iron was surrounded by a carbohydrate. The currently marketed parenteral iron preparations are considered equally efficacious but vary in molecular size of the nanoparticle, pharmacokinetics, and adverse reaction profiles.
In the present study, the available intravenous iron agents low molecular weight iron dextran (Cosmofer®, Infed®) sodium ferric gluconate (Ferrlecit®), iron sucrose (Venofer®), iron carboxymaltose (Ferinject®), ferumoxytol (Feraheme®) and iron isomaltoside (Monofer®) were compared with respect to particle size (GPC, DLS, TEM), structure (XRD), free iron content (dialysis), acid soluble iron and in vitro liberation of iron in plasma (Ferrozine method).
The particle size varied depending on the principle of the determination method (e.g. core vs. hydrodynamic diameter) but increased in the following order (TEM): sodium ferric gluconate < iron sucrose < LMW iron dextran < ferumoxytol ≈ iron isomaltoside. In case of iron carboxymaltose the cores tend to cluster and single cores are not definable. With respect to acid soluble iron the formulations showed clear differences in which sodium ferric gluconate and iron sucrose were the most labile complexes and ferumoxytol was the most stable followed by iron isomaltoside ≈ iron carboxymaltose ≈ iron dextran. A negative correlation between half life of iron liberation and surface to volume ratio of the complexes was observed. The liberation of iron into plasma demonstrated differences in the stability of the iron complexes in biological media and in principle but not in each case followed the rank order of acid soluble iron. In conclusion it can be shown that nanoparticular parenteral iron formulations differ which may in part explain their dosing recommendations and adverse reaction profiles.

T014

ENZYME-RESPONSIVE NANOPARTICLES FOR CARTILAGE TARGETING

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Inflammatory processes in synovial joints are characterized by upregulation of matrix degrading enzymes. Thereby, matrix metalloproteinases (MMPs) have predominant roles in both rheumatoid arthritis and osteoarthritis. Nanoparticles would be promising carriers for drug delivery into joints and even cartilage, since the dense cartilage matrix restricts access of larger vehicles [1]. Unfortunately the synovial fluid undergoes a continuous turnover so that even microparticles are rapidly cleared from the joint space within hours [2].
Here, we introduce an approach to use MMPs to specifically target and immobilize nanoparticles at such inflamed sites and show nanoparticle development and characterization. A PEG-coating protects our nanoparticles from unspecific tissue interaction and protein binding until the PEG-chains are cleaved by MMPs. Upon cleavage the surface characteristics of the particles change and allow them to bind to cartilage tissue by electrostatic interactions. As model particles gold nanoparticles were chosen. We synthesized collagenase-sensitive PEG-ligands by liquid phase peptide synthesis that allow for the attachment to gold via thiol-gold bonds. In order to achieve a compromise between protection and accessibility for the enzyme a mixed surface layer of cleavable and stable chains of different length was developed. As a surrogate for matrix degrading enzymes we used collagenase from Clostridium histolyticum. The sensitivity of the enzyme-responsive PEG-polymers was demonstrated. To further prove the feasibility of our approach we monitored the cleavage of the PEG-ligands by collagenase from a solid gold surface by surface plasmon resonance (SPR)-measurements. Enzymatic activation of PEG-coated nanoparticles could be observed by dynamic light scattering (DLS) experiments which revealed a decrease of the hydrodynamic diameter and a change of the zeta potentials. In contrast, the nanoparticles remained unchanged in the presence of the collagenase inhibitor EDTA. After the successfully demonstrated nanoparticle development, ongoing studies include interactions of the particles with cartilage tissue in vitro and the application in an in vivo rodent model.

[1]D.A.Rothenfluh et al, Nature Materials, 7, 248-254 (2008). [2]N.Gerwin et al, Advanced Drug Delivery Reviews, 58, 226-242 (2006)

T015

IN VIVO AND EX VIVO STUDIES OF PEG - PLA BLOCK COPOLYMER NANOPARTICLES FOR TUMOR VISUALISATION AND TREATMENT

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Nanoparticles (NP) have the potential to overcome multiple biological barriers and to deliver drugs selectively to tumor cells, but also for the application in tumor imaging for cancer diagnosis. It is known that the NP matrix (e.g. the polymer) and surface properties play an important role. It is reported that PEG can improve the *in vivo* behaviour. But also particle size variations are a critical factor concerning the *in vivo* fate caused by a rapid clearance of circulating NPs during systemic delivery thus avoiding undesirable accumulation in the body. In this study, the fate of different PEG_2-PLA_x block copolymer NPs was explored after i.v. administration. The near infrared dye DiR (invitrogen) was incorporated. This allows to measure the fate of the NPs through the whole body by non invasive multispectral *in vivo* fluorescence imaging [1]. Furthermore the HT29 and A2780 xenograft tumor models were used to explore the *in vivo* tumor accumulation of the NPs.
In vitro characterisations (A4F/MALLS, PCS and TEM) indicate narrow particle size distributions with the mean diameters in the lower nanometer range. Results of toxicity tests revealed that the NPs show no distinctive toxicity thus allowing injection to the mice. Detailed *in vivo* studies were performed to identify differences in distribution, accumulation and elimination behavior of different polymer ratios and particle sizes. The circulating NPs were detectable in the blood stream for over 4 hours. An accumulation in liver and spleen was detectable *in vivo*. High concentrations of the NPs in the HT29 and A2780 carcinoma tissues were observable already 6 h after injection. The intensity increased within the first 24 h. Different tests with these two carcinoma cell lines and also *ex vivo* tissues with confocal microscopy confirmed these results. *Ex vivo* studies were done to measure the maxima and total fluorescence intensities of different organs. This allows to identify possible excretion pathways by detecting slight accumulations in kidneys and intestine, which were not visible *in vivo*. Studies are ongoing in order to detect metastases by fluorescence imaging.
[1] Y. Jiang et al.: In-vivo studies on intraperitoneally administrated poly(vinyl alcohol), J. of Biomedical Materials Research Part B: Applied Biomaterials, 2010

T016

ROLE OF α-MODIFICATION ON PHYSICAL STABILITY OF LIPID NANOPARTICLES

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Introduction: The major aims in formulation development of lipid nanoparticles are a high drug loading capacity, physical long term stability and the ability to identify suitable formulations at an early stage of the development.
Experimental methods: A main reason of physical instability is the expulsion of drug over the time of storage. In this study differential scanning calorimetry (DSC) was used to compare the thermograms of drug loaded and non-loaded solid lipid nanoparticles (SLN, lipid matrix consist of one solid lipid), nanostructured lipid carriers (NLC, lipid matrix consists of a blend of a solid lipid and a liquid lipid) and their physical mixtures (i.e. non-homogenized lipid matrices of each system).
Results: The thermograms of homogenized and non-homogenized samples (physical mixtures) were not always similar. Thus only the homogenized samples (SLN and NLC with and without drug) could be used for analysis. For NLC only one melting point for the lipid was detected. This corresponded to the stable ß-modification. No changes over time were observed. In contrast to this in all SLN thermograms an additional lower melting point, corresponding to the α-modification of the lipid, was detected at the day of production. Over the time of storage (1 year) the amount of α-modification decreased. In parallel, for the SLN, drug expulsion was clearly detectable using light microscopy. No expulsion was observed for NLC. The results provide the first evidence, that a) the transformation of α-modification to β-modification causes drug expulsion, b) addition of liquid lipid can prevent the crystallization of the instable α-modification, c) DCS-thermograms of the physical mixtures cannot be used to screen for suitable lipid mixtures.
Conclusion: Drug expulsion during storage can be prevented if the lipid re-crystallizes without α-modification. This can be obtained if liquid lipid is added to a solid lipid matrix.

T017

DEVELOPMENT OF PRESERVED HIGHLY-LOADED ARGAN OIL NANOSTRUCTURED LIPID CARRIERS (NLC)

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Nanostructured lipid carriers (NLC) are used in many dermal cosmetic formulations, but are also in development for pharmaceutical products. In cosmetic industry, the cosmetic companies buy the NLC as concentrated suspensions from manufacturers of cosmetic excipients and actives, e.g. Dr. Rimpler GmbH/ Germany. The NLC concentrates are admixed to the finished products. For microbial safety reasons, these concentrates need to be preserved, but at the same time the preservatives can impair their physical stability. Therefore the compatibility with each formulation needs to be investigated, a systematic screening for preservatives performed. This was done in this study for Argan oil loaded NLC.
The consumer prefers preservative-free products. Therefore in this study substances were used, which do not need to be declared as preservative, but have preservation action. Several preservative systems were admixed to the developed formulation and the physical stability was monitored. In addition, the loading with Argan oil should be as high as possible, but the NLC still solid at body temperature. Therefore a screening of blends of argan oil with high melting lipids was performed to identify the blend with highest possible oil content (40% oil and 60% solid lipid in the blend).
Upon admixing ethanol 20% to the formulation, immediately particle aggregation could be detected using laser diffractometry (LD diameter 99% about 140 µm). The samples gelled after 24 hrs. On the other hand samples preserved with 10% propylene glycol did not show any change in particle size in comparison to the non-preserved formulation, measured after one day and 3 months storage. The mean particle size was about 230 nm (PCS) and the LD diameter 99% about 0.6 µm. Samples preserved with 5% pentylene glycol proved also stable after 3 months and did not show any change in particle size.
In this study it was shown that NLC with high Argan oil load can be produced. They were preserved successfully without affecting the physical stability of the suspension.

T018

IMPACT OF SALTS ON THE PARTICLE SIZE OF DISPERSED CUBIC PHASES

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Dispersed cubic phases, as they e.g. form in the system monoolein/poloxamer 407/ water after homogenization, are considered as potential drug carriers. Autoclaving is often performed after homogenization to improve the structure of the dispersions with regard to homogeneity and complete formation of the cubic phase [1]. In literature, the properties of poloxamer 407 are mentioned as potentially important factor for the temperature dependent formation of the cubic phase nanoparticles [2]. Since this point has previously not been studied in detail experimentally, this study aimed at elucidating the underlying mechanisms. Dispersions containing different concentrations of poloxamer 407 and monoolein, and in some cases 2.5 % glycerol for isotonization and/or 0.01 % thiomersal for preservation, were prepared from pre-equilibrated crude dispersions by high pressure homogenization, followed by autoclaving. Particles with a P-type cubic structure were detected within the resulting dispersions as expected. The presence of thiomersal, a surface active sodium salt, increased the particle size but did not change the cubic phase of the nanoparticles. In a system containing only poloxamer 407, glycerol and water, the addition of thiomersal lowered the cloud point of poloxamer 407. According to earlier investigations with similar systems, a comparable reduction of the cloud point can be achieved by the addition of salts [3]. This could be confirmed for the solution described above. With regard to the dispersions, there is a correlation between a reduction of the cloud point caused by thiomersal and a larger particle size after heat treatment. This can be attributed to a longer exposure of the dispersions to temperatures above the cloud point in comparison to the thiomersal-free dispersions, since only temperatures distinctly above the cloud point allow a fast and uniform particle growth. A very pronounced cloud point reduction by a high amount of salt like e.g. NaCl led to a collapse of the system. In conclusion, alterations of the cloud point of poloxamer due to the addition of excipients seem to have pronounced effects on the structural behavior of the considered dispersions. Clarification of these effects may help to better understand the formation of the cubic particles and to control the properties of the dispersions in the future.

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T019

ANALYSIS OF SUPERCOOLED SMECTIC NANOPARTICLES BY ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION

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Supercooled smectic nanoparticles have been introduced as carrier system for poorly water soluble drugs, particularly with respect to parenteral administration [1]. These nanoparticles are based on a strongly supercooled smectic phase of cholesterol ester(s). The liquid crystalline state may offer advantages over the liquid and the crystalline state of the lipid nanoparticle matrix. However, these dispersions are rather complex with regard to the colloidal structures involved: In addition to colloidal structures formed by the excess of stabilizer(s) (e.g., micelles, vesicles), two co-existing types of supercooled smectic nanoparticles have been observed in dispersions stabilized for example on the basis of phospholipids [1]. Asymmetrical flow field-flow fractionation (A4F) combined with multi-angle laser light scattering (MALLS) is a promising method for the analysis of such complex formulations due to its versatility, broad separation range (a few nm up to about 1 µm [2, 3]) and the possibility to obtain homogeneous sample fractions.

In a first approach, different formulations of supercooled smectic nanoparticles were analyzed by A4F/MALLS. In addition to size analysis, a homogeneous sample of supercooled smectic nanoparticles – where the majority of excess stabilizer has been removed – could be obtained by semi-preparative A4F and subsequent concentration by ultrafiltration and centrifugation. This purified sample was used to study the interaction of DSPE-mPEG-micelles with the lipid nanoparticles in order to obtain information about the possibility and efficiency of the preparation of PEGylated supercooled smectic lipid nanoparticles by a post-insertion process as described for lipid emulsions in the literature [4]. Our first results, however, indicate that post-insertion of DSPE-mPEG into smectic nanoparticles is not possible (or at least highly inefficient) by just mixing the lipid nanoparticles with the micelles.

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T020

NANOPARTICLES FOR THE ORAL DELIVERY OF IL-10 TO THE INFLAMED INTESTINE

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Interleukin-10 (IL-10) is a regulatory cytokine which has pleiotropic effects in immunoregulation and inflammation. It has been proposed as a potent anti-inflammatory therapy in inflammatory bowel diseases (IBD). So far, the clinical results of systemic recombinant IL-10 therapy in IBD were disappointing because of lack of efficacy at low doses and adverse effects at high doses. It is assumed that daily systemic application does not allow for efficient delivery to the sites of inflammation due to the short serum half life of about 1-3 hours. Thus, a local and sustained delivery to the inflamed intestinal mucosa appears to be a more promising approach, resulting in high local concentrations and avoiding systemic side effects. Drug delivery systems on the basis of nanoparticles may be promising as they selectively accumulate in the inflamed intestinal mucosa and have a longer transition time in the intestine compared to larger particles.

In this study nanoparticulate carrier systems for oral application and local release of IL-10 in the terminal ileum and the large intestine were prepared and characterised. In first studies bovine serum albumin (BSA) and fluorescein isothiocyanate-labelled bovine serum albumin (FITC-BSA) were used as model proteins.

T021

COMPARISION OF NANOEMULSION PREPARED BY HIGH PRESSURE HOMOGENIZATION AND ULTRASONICATION

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Nanoemulsions are a type of emulsion with monodisperse droplets typically in the range of 20-200 nm. The smaller droplet size makes them transparent or translucent. They are not thermodynamically but are kinetically stable[1]. Being non-equilibrium systems, they cannot be formed spontaneously instead requires high energy input, which can be achieved by using high-shear stirrers, high pressure homogenizers or ultrasound generators. The high energy input leads to deforming forces that are able to break the droplets into smaller ones, provided the Laplace pressure is overcome. High energy emulsification method has several advantages over low energy emulsification method. Those advantages included flexible control of droplet size and size distribution[2]. The mechanism for the formation of nanoemulsion using both the two energy sources involves the formation and collapse of cavitation bubbles filled with steam or gas. As a result of this cavitation, the dispersed droplets are disrupted following the formation of new droplets[3]. O/W emulsions intended for parenteral administration are designed for the incorporation of lipophilic drugs which exhibit poor aqueous solubility[4]. The size of nanoemulsion is affected by formulation and composition variables as well as by mechanical mixing condiitons[1].

The main objective of this study is to prepare nanoemulsion by using two different types of high energy input, namely high pressure homogenizer and ultrasound generator and compare their characteristic features on the basis of droplet size, zeta potential and stability. In future, the surface of nanoemulsions will be modified using different ligands or antibodies so as to study the cellular uptake of nanoemulsions by different cell lines. After successful uptake of drug loaded nanoemulsions, they will be finally targeted to the cells of interest, i.e., immune cells or tumour cells to study the anticancer activity of the loaded drug.

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T022

HIGHLY CONCENTRATED 40% I.V. NANOEMULSIONS FOR DRUG DELIVERY

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Poorly water soluble, lipophilic or amphiphilic drugs (e.g. amphotericin B) can be i.v. administered as o/w emulsions. These emulsions are on the basis of emulsions for parenteral nutrition, typically 10% or 20% oil content. However for some drugs this would still lead to an uncomfortable high injection or infusion volume. Therefore the production of ideally 40% i.v. emulsions is of interest and was investigated. The influence of production parameters (pressure, cycle number) on the resulting particle size and content of larger particles (tailing of size distribution) was studied.
The emulsions were produced by homogenization at 500, 800 and 1500 bar up to 5 cycles (Micron LAB 40). The particle size analysis was performed by laser diffractometry (LD) using a Mastersizer 2000 and photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (both Malvern Instruments, Malvern, UK).
Two cycles at 800 bar were sufficient to reach a size small as about 250 nm, polydispersity index 0.09 (PCS) for 30% emulsions. This is in the range of the size of emulsions for parenteral nutrition, i.e. acceptable. Applying only 500 bar production pressure required 5 cycles for a similar size. To make the production cost-effective, 800 bar is optimal. Increasing the pressure to 1,500 bar decreased the size only by 20 nm, that means it was not possible to reduce the number of cycles to one cycle by pressure increase. Increasing the oil concentration to 40% required 3 production cycles for reaching the same size range. No difference in size was found between 800 bar and 1,500 bar at 3 cycles for 40% emulsions, in addition no further size reduction could be achieved from 3 to 5 homogenization cycles. This can be explained by the loss of homogenization efficiency in the more viscous emulsions system – despite the same power density. The LD diameter 99% as measure for large droplets was about 0.6 µm for all emulsions.
In summary, 40% i.v. emulsions are feasible by a cost-effective production (800 bar, 3 cycles) still being of low viscosity due to the small droplet size.

T023

PRODUCTION AND CHARACTERIZATION OF O/W CONCENTRATED EMULSIONS STABILIZED BY PLANT PROTEIN

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Concentrated emulsion contains a very large amount of dispersed phase, thus it can significantly enhance the entrapment of pharmaceutical drug substances or other active agents. The aim of this work is to produce and characterize a concentrated emulsion, which is stabilized by soybean protein. The stability and particle size distribution for the concentrated emulsion were determined by a light scattering method. In order to prepare the concentrated emulsion, 100 grams of original emulsions (or pre-emulsion) with 30% w/w of Miglyol 812 and 70% w/w phosphate buffer were prepared separately using an ultrasonic probe (UP 75) and a high pressure homogenizer (Emulsi Flex®-C5). Soybean protein at the level of 1%, 2% and 3% w/w was applied as a stabilizer. It is important to control a pre-emulsion with the soluble soybean protein at pH=8, in order to get smaller droplets with stable interface protein films. The homogenizing time by the ultrasonic probe was 2 min. If the high pressure homogenizer was used the emulsion was pressed five times through the machine in order to produce the pre-emulsion. The pre-emulsion was centrifuged by a high speed centrifugation machine at 5400 min^{-1} for 30 min in order to receive the concentrated emulsion. The particle size distribution of the concentrated emulsion was determined by a Malvern Mastersizer-S. The concentrated emulsion was diluted with 10% w/w sodium dodecyl sulfate (SDS) and during the measurement with a light scattering method 5% w/w SDS was used instead of deionized water as the background to avoid the agglomeration between the drops. The size distribution of the concentrated emulsion produced by an ultrasonic probe is monomodal with a size-average of about 1.8 µm. In contrast, the concentrated emulsions prepared by a high pressure homogenizer show many peaks with the size-average of 2.1 µm which means that they have a broad size distribution.

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T024

DEVELOPMENT OF STABLE PERFLUOROCARBON-CONTAINING NANOEMULSIONS FOR THE USE IN $^{1}H/^{19}F$ MRI

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A new approach for the *in-vivo* detection of inflammatory processes by $^{1}H/^{19}F$-magnetic resonance imaging (MRI) using emulsified perfluorocarbons (PFCs) was established in two separate models of local inflammation in animal experiments. Due to the lack of any natural ^{19}F background in human or animal organisms, PFCs can serve as a positive contrast agent in $^{1}H/^{19}F$ MRI for the visualization of inflammatory processes[1]. Within these studies the monocyte-macrophage-system was identified as main carrier system taking the preparations to the injured tissues.
Next investigations deal with the development of homogeneous and stable nanoemulsions. Ostwald ripening is a well known phenomenon which occurs especially in inhomogeneous preparations and results in destabilization. To obtain nanoemulsions of definite sizes and narrow size distributions, after the high pressure homogenization a subsequent particle sizing by preparative size exclusion chromatography (SEC) was performed.
Further studies were conducted to decrease the side signals in MRI by increasing the specifity of uptake by the monocyte-macrophage system. It is well known that phagocytosis of particles depends on their size and charge. Therefore, the size and charge dependent phagocytosis was investigated using flow cytometry
Another approach to increase the specifity of the uptake is the coupling of specific ligands to the surface of the emulsion droplets. A well established coupling procedure for active targeting of liposomes is the sterol-based-post insertion technique (SPIT)[2]. For this purpose a conjugate of a specific ligand (e.g. antibodies, antibody-fragments) and an activated sterol-PEG_{1300} anchor were prepared and inserted into the lipid monolayer. The targeting efficiency was observed in *in-vitro* studies using flow cytometry.

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T025

INVESTIGATION OF THE CHEMICAL STABILITY OF SUPERCOOLED SMECTIC NANOPARTICLES

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Supercooled smectic cholesterol ester nanoparticles are under investigation as a new carrier system for lipophilic drugs [1]. The thermotropic liquid crystalline state of the matrix lipid combines a high viscosity with a certain mobility on the molecular level. This is expected to lead to advantages with respect to physico-chemical stability and drug loading capacity. Such nanoparticles can be prepared by high-pressure melt homogenization in the presence of emulsifiers. The nanoparticle surface can be modified by incorporating surface modifying agents into the aqueous phase before the homogenization process, e.g. PEGylated phospholipids to achieve steric stabilization. These PEGylated nanoparticles are a promising formulation with respect to small particle size, long-term stability against recrystallization and stability upon autoclaving [2]. Since previous studies showed an increase of the negative zeta potential and a decrease of the pH value in the dispersions during storage, the aim of this work was to investigate the chemical stability of the dispersions. As the systems are based on cholesterol esters and stabilized with phospholipids which are both susceptible to oxidation and hydrolysis, their stability was studied directly after preparation and during storage. Qualitative analysis of the phospholipids and the cholesterol ester was performed by HPTLC. In addition, HPLC was applied to quantify the cholesterol ester concentration. The influence of the chemical processes on the physical properties of the systems was observed by particle size and zeta potential measurements. Extensive hydrolysis during storage was observed in the dispersions solely stabilized with phospholipids, leading to a pronounced decrease in pH. In the systems additionally containing sodium glycocholate degradation occurred only to a very minor extent. The phospholipid hydrolysis could be reduced by adding TRIS buffer (10 mM, pH 7.4) into the aqueous phase before the preparation process. Extensive phospholipid hydrolysis seems to promote the degradation of cholesteryl myristate, as detected in several dispersions during storage.

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T026

SOLID LIPID NANOPARTICLES (SLN) AS A TOOL FOR THE ENHANCEMENT OF THE BIOAVAILABILITY OF CURCUMIN

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Dried and powdered rhizoma of turmeric, *Curcuma longa*, is a major ingredient of curry powder. This spice finds widespread use in South Asia. The characteristic yellow colour of turmeric is caused from phenolic compounds, so called curcuminoids. The principal curcuminoid is curcumin (Diferuloylmethan). Numerous scientific articles have reported about the beneficial effects of curcumin (1,2). One point of particular interest is its anticancer activity. However the main problem that limits the application of curcumin as a pharmaceutical is its low oral bioavailability (2). One possible pathway to increase bioavailability is the development and application of nanoparticles. In the present work curcumin was incorporated in solid lipid nanoparticles (SLN) and nanoemulsions.
The mixture for the SLN and nanoemulsion contained 10% (w/w) lipid (glyceroltrimyristate, glyceroltristearate, castor oil), 2.5% emulsifier Poloxamer 188 (w/w), and 87.5% (w/w) of distilled water. The components were mixed at room temperature and heated up to 80-85 °C. After the lipid was melted an emulsion was formed using an ultra-turrax (IKA, Staufen, Germany) for 5 min at 14000 rpm. The hot premix was processed through a Stansted high-pressure homogenizer (Stansted Fluid Power Ltd., Stansted, UK). The starting pressure was set at 50 MPa and was increased every three cycles up to 100 MPa. The nanodispersions were cooled down slowly to room temperature, filled into glass vessels and stored either at 22 °C or at 8 °C. In further experiments curcumin in the range of 20-75 mg was embedded into the lipid phases and processed as described above. The mean particle size of the SLN preparations was at 150 nm whereas the nanoemulsion showed a mean particle size of 300 nm. The shape of the particles was investigated by transmission electron microscopy (TEM). The particles showed an anisometric shape. An in vitro prediction of the biofate of the particles and curcumin was gained by carrying out a pancreatin-assay. The stability of the incorporated curcumin was tested by incubating the preparation over several days under different conditions.
Our experiments show that high-pressure homogenization is a valuable method for the production of nanoparticles. It was possible to produce stable particle dispersions and to incorporate curcumin. Furthermore characterization of the particles using e.g. DSC, XRD, EPR, a4F and NMR is ongoing.

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T027

QUANTITATIVE IMAGING – A NEW APPROACH TO QUANTIFY NUCLEAR IMPORT OF LIPOPLEX-DELIVERED pDNA

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Cationic lipids spontaneously bind, condense and coat DNA resulting in the formation of lipid/DNA complexes, so-called lipoplexes [1]. These complexes transduce plasmids into cells causing expression of the genes (transfection). Low levels of transfection circumvent therapeutical efficacy of non-viral strategies, whereby nuclear accumulation of the plasmid DNA is among the major obstacles of these non-viral delivery systems [2].

This project aims to analyse nuclear transport of lipoplex-released pDNA as a potential transfection barrier in two cellular models, A-10 SMC and MDCK II.
A novel strategy called “quantitative imaging” is applied to study nuclear import of lipoplex-delivered pDNA, implying a combination of confocal laser scanning microscopy and image-based computer analysis using the open source software CellProfiler [3]. This strategy has already been used in former work to quantify the intracellular dissociation of lipid/DNA complexes followed by FRET analysis [4]. Here, this technique enables to track cy3-labeled complex DNA inside the cell and to quantify the amount of nuclear-accumulated complex released pDNA.

These studies reveal nuclear entry of the pDNA to represent a decisive transfection barrier in MDCK II cells. The investigated cellular models differ strongly in the amount of nuclear-accumulated complex DNA: nuclear transport is by far more efficient in A-10 compared to MDCK II cells.

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T028

PREPARATION OF EMULSIONS AND SOLID LIPID PARTICLES BY DIRECT MEMBRANE EMULSIFICATION

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Aqueous lipid emulsions and solid lipid particles with particle sizes in the nano- and micrometer range are being investigated intensively as drug delivery systems. They are usually prepared by high energy dispersion techniques like high-pressure homogenization. Homogenization generates high shear forces and may thus not be suitable for the processing of sensitive substances, like e.g. proteins. Therefore, this study investigates the possibility to prepare dispersions of lipid particles by direct membrane emulsification as an alternative low energy and low shear method. Direct membrane emulsification is an established technique for the preparation of monodisperse microparticulate emulsions. In this process, the liquid lipid phase is forced by low pressures through the pores of a membrane into the aqueous continuous phase, which is recirculated, e.g. by agitation with a stirrer, to facilitate droplet detachment. The lipid droplets grow at the pore openings of the membrane surface and are stabilized by emulsifiers present in the continuous phase. When the droplets reach a certain size they detach from the membrane. Solid lipid particles are usually processed above the melting point of the lipid and subsequently solidified by cooling below the recrystallization temperature. In the present study, the influence of the emulsifier (sodium dodecyl sulfate, polysorbate 20, sorbitan monooleate), the type of the lipid phase (medium chain triglycerides, soybean oil, trimyristin) and the pore size of the SPG (Shirasu porous glass) membrane on the particle size of the resulting dispersions was investigated. The particle size distribution was measured by submicron enhanced laser diffractometry and additionally with polarization microscopy. The particle size was primarily controlled by the pore size of the membrane. Typical ratios of mean pore size to mean particle size were in the range of 1:3 to 1:4.The influence of the surface properties of the glass membrane and the additional use of ultrasound were also under investigation. Hydrophilization of the membrane by plasma treatment and sonication during emulsification both reduced the comparatively long production time, which is a main disadvantage of direct membrane emulsification. Also the pressure required for droplet formation was lowered. This might be an advantage for the preparation of nanoscaled systems, because an increasing pressure was required and the production times increased with decreasing pore size.

T029

UTILIZATION OF CUSTOMIZED MICROCHANNEL GEOMETRIES FOR SOLID LIPID NANOPARTICLE PRODUCTION

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Solid lipid nanoparticles (SLN) are commonly produced by means of high pressure homogenization of a dispersion of molten lipids in a continuous aqueous phase. To achieve adequately small particle sizes and a narrow distribution of these, multiple passes through the homogenization valve are necessary. Thus homogenization is a discontinuous batch process. This holds for all other essential process steps: pre-emulsification, dissolving or dispersing APIs in the lipid matrix or aqueous phase, and crystallization. To establish all these process steps in one overall micro system, a continuously passed through setup is required. This facilitates low dead volume and small production scale, as desired for formulation screening. However, homogenization by a single passage poses a major challenge to the microchannel geometries. High efficiency in droplet break-up is required.
Customized micro structures were at first manufactured in silicone by wet-chemical etching. Different flow regimes (shear, elongational and turbulent flow) were applied by generally differing design approaches. Minor geometric changes within these design groups were carried out to elucidate their influence on product qualities. These were preliminary demonstrated using an emulsion (5 % Miglyol®812, 3 % Solutol® HS 15 in water). Orifice-like micro channel structures, applying high elongational stress to the product flow, were identified superior by producing small particle sizes (down to about 450 nm for one passage at 300 bar) and showing comparably low volume flow rates.
The manufacture of *solid* lipid nanoparticles necessitates higher pressures up to 1500 bar for single-pass production and elevated temperatures. The micro component substrate was changed to steel structured by µ-EDM (electrical discharge machining) to overcome these obstacles (silicone micro components rupture above 400 bar). SLN [1] with a median particle size of about 120 nm were produced by one single passage using an 80 µm orifice with a length of 600 µm.

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T030

CHARACTERISATION OF HIGH PRESSURE DISPERSION PROCESSES IN DIFFERENT MICRO CHANNEL GEOMETRIES

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High pressure dispersion in micro channels has several characteristics which recommend this method especially for pharmaceutical applications, e.g. a narrow residence time distribution, the possibility to use small educt batches and a relatively accurate adjustment of the induced stresses with a good reproducibility. Due to the small dimensions and the corresponding small volumes the micro systems have a low inertia which enables the application as screening instruments for the processing of valuable active pharmaceutical ingredients. Nevertheless abrasion of the micro channels and depositions, which can lead to blockages or contamination of the product, pose big challenges.
Two types of micro channels have been analyzed: silicon micro channels covered with a glass plate enabling an optical access for the flow measurements (µPIV) up to an entrance pressure of 500 bar and stainless steel micro systems enabling a pressure drop of up to 2300 bar. In order to characterize the high pressure dispersion process, flow analysis by means of “Computational Fluid Dynamics” simulations (CFD) in combination with “Micro Particle Image Velocimetry” (µPIV) and dispersion experiments with inorganic nanoparticles were carried out. Four channel geometry types, straight, Z-, Y- and orifice channels were analyzed. Dispersion experiments exposed a clear hierarchy regarding the dispersion efficiency of the different geometry types at constant pressure drops. The results show that the geometries as well as the dimensions of the micro channels influence the dispersion efficiency, the appearance of blockages and the amount of abrasion. With CFD-simulations and flow measurements (µPIV) areas of low velocities or of backflow connected with the risk of depositions and the occurrence of cavitation were identified. The CFD simulations are also conducted to get a better understanding of the stress field by solving the turbulent flow fields of the different micro channels and using these for a stationary particle tracking. Based on these particle pathes the elongational, shear and turbulent stresses were calculated.

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T031

DISPOSABLE PDMS MICROBIOREACTORS WITH INTEGRATED ONLINE ANALYTICS FOR BIOTECHNOLOGICAL SCREENING

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Considering both the high quantity of existing - but still unknown - microorganisms and the rapid progress of genome sequencing techniques, the development of cultivation platforms aiming at reliable high-throughput screening for bioprocess development is still a challenge. Small volume cultures offer the advantageous combination of a global reduction in experimental costs and time with simultaneous process enhancement. The latter is related to the fact that microenvironments allow precise control of procedural conditions in combination with an increased flexibility of parameter screening. With regard to high-speed drug screening, in vivo- or in vitro-like microenvironments for cells also play an important role because novel drug formulations are often limited in volume. During screening, online monitoring of different physical, chemical or biological parameters is indispensable in these microenvironments, since elaborate offline analytics are often limited due to small available sample volumes in rapidly changing microcultures. Using microtechnologies, innovative screening tools can be fabricated that simultaneously allow the integration of fluidic structures with electrochemical and optical elements for culture control and monitoring. By use of soft lithographic techniques in combination with poly(dimethylsiloxane) (PDMS), inexpensive disposable microchips can be produced with transparent and biocompatible characteristics. The microbioreactors (MBR) presented here are based on glass substrates (optionally structured with metallic electrodes) that are covalently bonded to a PDMS layer. This PDMS layer features the desired reactor design and structures for different optical interrogation approaches. All the MBRs include different types of online analytics to monitor retention time, dissolved oxygen concentration, pH, cell morphology or optical density. Cultivations of yeast cells (*S. cerevisiae*), spores (*A. ochraceus*) and primary human endothelial cells demonstrate the successful performance (such as proven scalability when compared to laboratory-scale bioreactors) of these highly integrated biomicrodevices for versatile application as disposable screening tools.

T032

DETERMINATION OF LIPOSOMES WITH DIFFERENT DRUGS

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Liposomes have been widely used in pharmaceutical field as transdermal and parenteral application. Liposomes are an alternative system for reducing the toxicity associated with drug.
The aim of this research work was to study the new formulation based on liposomes with different drugs which can be used as nano drug delivery systems. In our project the liposomes were prepared by lipid film hydration technique. A lipid film of different Phospholipon-types was prepared in a vial by dissolving the lipid in a mixture of chloroform and methanol (2:1, v/v) followed by removal of the organic solvent by a vacuum drying cabinet at 40°C for 24 h. Prior to the measurement by X-ray scattering, the lipid films were dissolved in sterile pure water at different concentrations of a model drug. In order to determine some properties of liposomes, e.g. shape, diameter and repeat distance (long spacing and water layer) the X-ray and light scattering as well as electron microscopy were applied. X-ray scattering based on synchrotron radiation allows a high resolution surface or interface sensitive structure analysis. Therefore the X-ray scattering technique from the synchrotron source at the beamline BW4 and B1 at HASYLAB, DESY, Hamburg was applied to determine the significant peaks of the scattering pattern, which can give information about the different formulations. The information about the nanostructure of the different formulations by various measuring techniques will allow us to formulate the better drug delivery system and to understand the mechanism of action of different composition inside the formulation.

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T033

OPTIMIZING SHELF LIFE OF DOXORUBICIN LOADED LIPOSOMES BY LYOPHILIZATION

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The stability of aqueous liposome preparations is influenced by hydrolysis of the phospholipids being part of the formulation [1]. One possibility to circumvent this problem is the storage of liposomes in the dry state. Freeze drying of a liposome dispersion can be applied to achieve a porose lyophilisation cake which can be easily reconstituted when needed [2]. The removal of water affects the liposome integrity because of its spacer-function between the vesicles, resulting in size changes and leakage of the encapsulated compound [2]. Therefore, lyoprotectants like disaccharides can be added to protect liposomes against freezing and thawing stress [2].
HSPC (hydrated soy phosphatidylcholine)/cholesterol liposomes were prepared by the lipid film method and subsequently loaded with doxorubicin (DXR) using the remote loading method. The encapsulation efficiency (EE) of DXR was detected fluorimetrically on the LS 50B, Perkin Elmer.
For the freeze drying process half of the samples were frozen in the -80°C deep freezer whereas the other half was cooled down in the freeze dryer using an Alpha 2-4 (Martin Christ, Osterode, Germany). After complete freezing the samples were united in the freeze dryer and the primary drying lasted 72h with a starting temperature of -35 °C followed by the secondary drying for 6 h at 30 °C. The lyophilized products were stored at 4 °C and characterized regarding EE and size changes. Depending on the amount of sucrose the EE varied showing the best results with higher concentrations of sucrose. Regarding size changes before and after freeze drying the nontreated liposomes seem to be more stable.

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T034

BORON-LIPIDS IN LIPOSOMES: LIPOSOME/CELL INTERACTION AND LIPID EXCHANGE

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In Boron Neutron Capture Therapy (BNCT) of tumours, ^{10}B-containing molecules within the tumour are subjected to neutron rays. The resulting α- and ^{7}Li-particles act as damaging agents to the adjacent tumour cells. Liposomes might be helpful in transporting the molecules to the tumour site.

In this study, SAINT-like lipids which contain negatively charged closo-dodecaborate clusters as polar head groups and feature varieties in alkyl chain lengths as well as linker moieties to the boron cluster [1] are used as main components in the formulation of liposomes. The cellular association of the liposomes and the exchange of lipids between membranes (e.g. liposomes) is of particulate interest in this project.

For cell association studies, Kelly cells were incubated (37 °C or 4 °C) with liposomes consisting of SPC (soy phosphatidylcholine), cholesterol and the respective boron-lipid in equimolar amounts along with Rhodamine-PE as fluorescence label and optionally 5 % DSPE-PEG-2000 (PEGylated distearoyl-phosphatidylethanolamine). Flow cytometry analysis showed cellular association of the boron-liposomes in the range of 30 to 80 % at 37 °C, and below 20 % when incubation was performed at 4 °C. This indicates that an active internalization of the liposomes takes place. For lipid alkyl chain lengths of C_{14} and C_{16} a decrease in association can be observed for preparations containing DSPE-PEG-2000.

Boron-lipid exchange might occur with tissue membranes (e.g. blood vessel endothelium) before arrival of the liposomes at the designated target tissue. Therefore, the analysis of the exchange of lipids between liposomal bilayers was performed by means of free-flow electrophoresis [2]. First results indicate a correlation of exchange rate and diminishing chain length when using similar lipids.

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T035

ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION FOR THE CHARACTERIZATION OF LIPOSOMES

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In asymmetrical flow field-flow fractionation (A4F) a laminar flow and a lateral cross flow is applied in a channel for sample fractionation in contrast to conventional chromatographic methods using a stationary and a mobile phase. By combining the A4F system with a multi-angle light scattering detector (MALLS) size determination of colloids at each elution time becomes possible and accurate determination of size distributions can be achieved. Asymmetrical flow field-flow fractionation is thus a promising method for size determinations of colloidal drug carrier systems like liposomes, as one of the most important parameter is carrier size and size distribution which affects circulation time in-vivo as well as biodistribution and targeting abilities.

In the present study, liposomes composed of DPPC/DPPG (9:1 w/w) were analyzed by A4F/MALLS. Drug-free liposomes and liposomes loaded with different lipophilic drugs were prepared in 5 % glucose solution by extrusion. The influence of the osmolarity of the carrier liquid (pure water, sodium chloride solutions (25, 50, and 100 mM) and an isotonic glycerol/water mixture) on the size of the liposomes was evaluated. To obtain information about liposome and drug recovery different radioactive markers (either ^{3}H or ^{14}C) were incorporated into the liposomes.

Reproducible results were obtained under all fractionation conditions. Vesicle size was, however, affected by the carrier liquid with largest sizes measured in pure water. Nevertheless, no indication of vesicle destabilization or disturbed elution behavior was observed. Interestingly, liposomes loaded with temoporfin (a water-insoluble, highly hydrophobic photosensitizer) were less sensitive to osmotic swelling than the drug-free liposomes. Whereas liposome recovery (lipid marker) was close to 100 %, recovery of incorporated drugs was less in all cases and strongly dependent on the partition coefficient of the drug. Recovery of temoporfin (logP ~ 9.597) for example was about 80 % whereas recovery of corticosterone (logP ~ 1.758) was only 2 %.

T036

WHEAT GERM AGGLUTININ MODIFIED LIPOSOMES FOR IMPROVEMENT OF PHOTODYNAMIC ANTIBACTERIAL THERAPY

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Abstract:
Recently, photodynamic antimicrobial therapy (PACT) attracted a lot of attention as a promising treatment modality to eradicate bacteria, especially against antibiotic-resistant species. In this study, a generation II photosensitizer, temoporfin, was incorporated into liposomes to increase its solubility, and the liposomal surface was modified with a lectin, wheat germ agglutinin (WGA), aiming to improve the targeting delivery of Temoporfin to bacteria. In use of a functional phospholipid, DSPE-PEG_{2000}-NHS, WGA was successfully and conveniently conjugated to liposomes, proved by gel electrophoresis. Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* were used as the model of gram-positive and gram-negative bacteria, respectively. The delivery of temoporfin to bacteria was confirmed using fluorescence microscopy, while the delivery efficiency of different formulations was compared using flow-cytometry, proving that the WGA modified liposomes delivered more temoporfin to MRSA and *Pseudomonas aeruginosa* compared to unmodified liposomes. In the photodynamic inactivation test, the WGA modified liposomes eradicated all MRSA and increased the bactericidal efficacy significantly against *Pseudomonas aeruginosa*, showing obvious improvement of PACT. Therefore, the WGA bearing liposome is a potential modality for PACT against antibiotic-resistant bacteria, particularly of great importance for local microbial infections.

T037

GRAPHITE FURNACE AAS AS A QUANTITATIVE ANALYTIC METHOD FOR DETERMINATION OF INTRALIPOSOMAL ARSENIC TRIOXIDE

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The aim of the PhD project is the effective encapsulation of arsenic trioxide (ATO) as an antineoplastic drug[1] into liposomes combined with an active targeting against GD2, as a specific marker for neuroblastoma cells. The first critical challenge for the determination of the intraliposomal arsenic trioxide amount is to establish a suitable quantitative analytic method within a range of 5-10 µg/l. According to results of Chen et al. 2006[2], ATO could be effectively encapsulated via a remote loading mechanism. Previously encapsulated nickel acetate forms an unsoluble precipitate with ATO resulting in high encapsulation efficiency (98%).

A quantitative analytic method for arsenic trioxide was affected by a complex matrix including liposomes, NiIIAc and isotonic HEPES buffer. Graphite furnace atomic absorption spectroscopy (GF-AAS) is a suitable quantitative analytic method with regard to high sensivity level even with an elevated salt burden. In GF-AAS, a single drop (20µl) of sample to be analyzed is positioned on the platform of a graphite tube. In a multi-step temperature program, the drop evaporates, matrix is then removed through pyrolysis, molecules are atomized and absorption of these atoms is detected. Following optimization of length and temperature of the evaporation, pyrolysis and atomization phase, the detection and quantification limit was determined via the calibration line method. Within a range of 2.5-25 µg/l a sixfold measurement was performed, and results analyzed concerning relative standard deviation and linearity. It could be shown that a concentration of 5 µg/l could be securely determined with a RSD less 5% and linearity over the entire range. Different lipid compositions were tested in order to find the optimal mixture for highest encapsulation efficiency.

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T038

PHOSPHOLIPIDS AS POTENT IN-VITRO P-GP INHIBITORS

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Introduction
Oral drug administration is mainly regulated by the step of intestinal absorption. For various substances uptake is strongly superimposed by an active efflux back into the lumen via ATP-driven pumps like the P-glycoprotein (P-gp), significantly decreasing their bioavailability.
P-gp inhibitory effects can be determined via in-vitro transport studies and the calcein accumulation assay (CAA) using CaCo2, as well as MDCKII-mdr1 cells. Former results indicate that particular synthetic phospholipids (PL) display a P-gp inhibitory potential.
The aim of this study is to identify further potent PL species and to investigate their mechanism of inhibition by varying diverse experimental parameters.
Experimental Methods
Cell culture: CaCo2 and MDCKII-mdr1 cells were routinely maintained in supplemented DMEM. Cells were grown for either 21 days in Transwell® plates in case of transport studies, or for 8, resp. 4 days (MDCKII-mdr1) in 96-well plates in case of CAA.
Lipid formulations: PL were applied as micellar or liposomal formulations.
Transport studies: CaCo2 cell layers were pre-incubated with lipid and digoxin (^{3}H-labeled) was added apically for absorptive or basolaterally for secretory studies, respectively. Monolayer integrity was determined via transepithelial electrical resistance (TEER) measurements and the ratio of the apparent permeability coefficients (P_{app}) of both directions displayed P-gp effects.
Calcein Accumulation Assay: After pre-incubation with lipid or Verapamil as a positive control the intracellular accumulation of the fluorescent dye calcein indicated P-gp inhibition.
Results
C12-phosphatidylglycerol, C6-phsophatidylserine and various unsaturated symmetric and asymmetric phosphatidylcholines (PC) induced a significant, concentration-dependent enhancement of net drug absorption in the CaCo2-transport studies. Using CAA C10-PC displayed a higher P-gp inhibiton than Verapamil depending on the pre-incubation time, whereas cis-22:6-PC showed a pronounced transporter selectivity.

T039

SCALE DOWN ABILITY OF ASEPTIC DRUG NANOCRYSTAL PRODUCTION

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Drug nanocrystals are an approach for the formulation of poorly soluble drugs, including parenteral injections (i.v., i.m.) [1]. For injectables, aseptic production is typically required because terminal sterilization is in many cases not feasible. The aseptic process requires equipment suitable for aseptic processing. In lab scale, high pressure homogenizers are more convenient than bead mills. Previously an aseptic production line was established based on an APV Gaulin LAB 40 with a batch size of about 40ml. However, even when producing a low concentrated 1% nanosuspension, this requires 0.4g material per batch. This is far too much for very expensive drugs, and new chemical entities of very limited availability, esp. in a systematic screening. Therefore the scale down ability was investigated by using an Avestin B3 with about 3ml batch size, reducing the amount of drug needed by a factor >10. The aims of the study were to assess a) how efficient the B3 is compared to the LAB 40, and b) if comparable results can be achieved with both homogenizers, i.e. if previous experiences can be used for the B3. Several actives with antioxidative capacity, and with potential use in supportive cancer treatment (curcumin, hesperetin, hesperidin), were used to produce nanosuspensions with both homogenizers. Production was performed applying a pre-milling with increasing pressure, and 20 homogenization cycles at 1,500 bar. Size was analyzed as a function of cycle number by photon correlation spectroscopy (Zetasizer Nano ZS) and laser diffractometry (Malvern 2000, both Malvern Instr., UK). The result depended very much on the type of active. For hesperedin, a similar PCS size was obtained (about 280 nm), hesperetin and curcumin were distinctly larger when using the B3 (about 546 nm versus 378 nm respectively 925 nm versus 633 nm with LAB 40). In some productions a small size identical to the LAB 40 was obtained, but production reproducibility was insufficient (hesperedin). This is attributed to the lack of precise pressure control, whereas reproducibility with the LAB 40 was good. In addition, often the size decayed slower with increasing cycles. In summary: for nanocrystals the B3 appears suitable for first feasibility runs, but – in contrast to nanoemulsions - not optimal for screening of production parameters.

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T040

BIOACTIVITY AND CONFORMATIONAL STUDIES ON CYTOKINE-COATED MICROCRYSTALS

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The rapid introduction of an aqueous buffered protein solution in which carrier material is dissolved into a water-miscible organic solvent such as propan-2-ol or 2-methyl-2,4-pentanediol leads to the formation of protein-coated microcrystals (PCMCs). Due to the immediate removal of the water no crystal growth can occur after nucleation for kinetic reasons. Hence, the protein as well as other components dissolved in the aqueous phase precipitate as fine particles in the amorphous form. After the precipitation process the resulting PCMC suspension can be concentrated and finally be dried via supercritical fluid extraction (SFE) using supercritical carbondioxide. The PCMCs can either be utilized as fine and well flowing powder or be resuspended in another nonsolvent system [1].
A precedent study had focused on a formulation screening to assess the applicability of this technology to a non-glycosylated cytokine [2]. One very promising formulation was chosen to investigate in depth bioactivity and protein structure of the cytokine. The analytical focus was placed on a cytopathic effect assay as bioactivity test as well as on the characterization of the protein structure via fluorescence, 2nd derivative UV and FT-IR spectroscopy.
Overall, the cytokine´s bioactivity was entirely preserved after the PCMC precipitation process. In this respect, the choice of the reconstitution medium turned out to have a tremendous effect concerning ionic strength and pH. The reconstitution media that combined an acidic pH and a low ionic strength led to optimal recovery of bioactivity. Bioactivity of cytokine formulated as PCMC were equivalent to standard lyophilized formulation. Furthermore, no structural changes of the protein could be detected by the spectroscopic methods comparing the starting material prior to precipitation and the PCMC powder after reconstitution. Thus, the PCMC precipitation process had no deleterious effect on the very cytokine and was successfully applied whilst preserving bioactivity and protein conformation.

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T041

THE USE OF DOE TO OPTIMIZE PROCESS PARAMETERS FOR A NOVEL PRODUCTION METHOD FOR NANOSUSPENSIONS

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Modern active pharmaceutical ingredients (APIs) show in most of the cases poor water solubility, which causes an inadequate dissolution rate and therefore a low oral bioavailability. Particle size reduction (PSR) with high pressure homogenization (HPH) is a suitable method to enhance the bioavailability of these APIs. The achievable particle size is depending on certain compound properties, such as crystallinity, hardness and morphology. In some cases it is difficult to obtain small particles. To solve this problem a combinational PSR method (FD-HPH) was developed, which is a combination of freeze drying (FD) (bottom-up) with HPH (top-down). The FD step modifies the drug structure and the HPH nanosizes the particles. First experiments have shown a relation between the FD conditions and the final particle size. Both the API concentrations as well as the organic solvent composition influence the porosity and the crystallinity of the drug during lyophilization. To properly analyse the influence of these parameters studies were conducted according to design of experiments principles. The model compound glibenclamide was dissolved in organic solvents (mixtures of DMSO and TBA) within different concentrations. The obtained API solutions were snap-frozen with liquid nitrogen and freeze dried. The outputs were characterized using SEM, DSC and XRPD and subsequently homogenized at high pressure using a Micron LAB 40 (APV-Gaulin) homogenizer. The nanosuspensions were characterized using PCS and LD for average particle size and distribution. Significantly smaller drug nanoparticles could be produced by using optimized process conditions. After 20 homogenization cycles with the modified API (high TBA content and low API concentration during FD, amorphous structure) the particle size was very small: 187 nm (PCS z-ave) and 0.146 µm (LD 50%). On the contrary, with unmodified API the results were 772 nm (PCS z-ave) and 0.520 µm (LD_V 50%). It was shown, that the structure modification of the drug by means of FD can significantly improve the particle size reduction effectiveness of HPH. The solvent and the drug concentration used for the FD need to be selected carefully. For this purpose design of experiments (DoE) is a very useful tool.

T042

INJECTABLE EXTENDED RELEASE LIDOCAINE SMARTCYSTALS FOR DERMAL APPLICATION

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Water soluble anesthetics (e.g. lidocaine HCL) are rapidly cleared from the application/injection site by diffusion. To obtain an extended release formulation for dermal and later on injectable use, the poorly water soluble lidocaine base was formulated as nanocrystal suspension (nanosuspension). Crystals provide a prolonged release. Sufficiently high drug levels can be generated by adjusting the surface (size) of the nanocrystals as one of the dissolution controlling factors. Lidocaine base nanosuspension 5% (w/w), stabilized with 1% Plantacare 2000 UP, was tried to be produced by high pressure homogenization (HPH). This resulted in a suspension with relatively large crystals showing pronounced sedimentation and caking tendency. Obviously the high energy milling process was not suitable for this formulation composition.
Therefore, as alternative a low energy smartCrystal production process was used, the combination technology (CT) of pearl milling followed by low pressure HPH. Lidocaine was produced using a PML 2 pearl mill (Bühler AG, Switzerland) for 3 hours followed by high pressure homogenization at a low pressure of 300 bar. The produced smartCrystals were characterized by photon correlation spectroscopy (PCS, Zetasizer Nano ZS (Malvern Instruments, UK)), laser diffractometry, (LD, Mastersizer 2000 (Malvern Instruments)), and light microscopy (Orthoplan, Germany) to check for presence of aggregates.
Particle size reduction was observed as function of milling time in the small size milling chamber. The smallest achieved mean particle size for the lidocaine smartCrystals was 244 nm with a polydispersity index of 0.173. LD and light microscopy confirmed absence of aggregates and showed uniform distribution of crystals. From this, the low energy CT process is suitable, and Plantacare 2000 UP efficient in stabilizing the nanocrystals and preventing aggregation. The nanosuspension had a homogenous, nice, uniform looking appearance. Exchanging the Plantacare against GRAS accepted stabilizer for injection (e.g. lecithin, Tween 80) opens the perspective for an injectable prolonged release formulation, of interest e.g. after surgery.

T043

PREDICTION OF PARTICLE FORMATION AFTER STIR STRESS OF AN IGG1 SOLUTION

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The discussion about protein aggregation is becoming more and more important in the field of biologicals which need to be administered parenterally. With reference to this it is interesting to evaluate whether aggregation and particle formation can be described and predicted by fitting data to a theoretical model.
One typical stress factor for biopharmaceuticals during manufacture storage and handling is mechnical stress. We studied stirring stress, as upscaling of stirring processes is a challenging task and on the other hand provides an excellent opportunity to apply mathematical modelling. Factors to be considered are: stirring speed, vessel size, formulation.

Using computational fluid dynamic simulations software (STAR-CCM+; CD Adapco, Nuremberg) the stirring process at three different stirring speeds in injection bottles using a magnetic stir bar was simulated to get information about the applied stresses in the liquid phase. In parallel, lab experiments were carried out: IgG1 solutions were stressed by stirring and measured afterwards using light obscuration to determine the particle count in size classes >1µm.
Stressing with other stirring speeds than used within the simulation experiment, the particle counts were found to properly fit into the simulated maximal stress tensor magnitudes.
We found that, keeping all conditions the same except stirring speed, the count of particles >1µm and >2µm can be well predicted. However, count of particles >10µm and >25µm as tested according to USP <788> can not be predicted using the applied software model.

Such results raise the question whether new pharmacopoeial specifications regarding particle counts in biotec-parenterals are needed to cover particles in the range 1-10µm, as they are obviously behaving different than particles >10µm and >25µm.

T044

INVESTIGATIONS ON LABELING OF $AL(OH)_3$-GEL FOR MAGNETIC RESONANCE TRACKING

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As early as in 1926 Glenny et al. reported the use of aluminium compounds as adjuvants in vaccines. Since that time aluminium hydroxide ($Al(OH)_3$) and aluminium phosphate ($AlPO_4$) have most commonly been used[1]. Nevertheless, the mode of action could not be ascertained in detail up to date. For a long time a depot effect was accepted as an explanation, but in recent years it became clear that this is not the sole cause [2]. A novel method to investigate the fate of $Al(OH)_3$ *in vivo* could be magnetic resonance imaging (MRI). As $Al(OH)_3$ is not directly visible in MRI, labeling with superparamagnetic iron oxide particles might be feasible. Here we used ferucarbotran particles (Resovist®, Bayer Schering Pharma AG, Germany) for the generation of complexes with $Al(OH)_3$. Resovist® is a commercially available MRI contrast agent for imaging of liver lesions consisting of carboxydextran coated iron oxide nanoparticles as an injectable solution. Since it is a FDA-approved contrast agent, the potential for clinical trials is given.
For labeling a colloidal suspension of $Al(OH)_3$ (Alhydrogel®, Sigma-Aldrich, Germany) was merged with ferucarbotran particles in varying ratios of iron and aluminium. The formed complexes were characterized regarding their size by dynamic light scattering and laser diffraction measurements. Their zeta potential was determined by electrophoretic light scattering measurements. To investigate the stability of the aggregation the complexes were centrifuged after one, three and five days of incubation in different media. The pellet and the supernatant were analyzed concerning iron content using atomic absorption spectrometry. When adding excessive adjuvant Resovist® was completely bound. The adsorption was stable for at least five days.
The first investigations showed that $Al(OH)_3$ was labeled with Resovist® simply by mixing. The adsorption probably resulted from electrostatic interactions due to opposing zeta potential of both components. The results of stability investigations are encouraging. The identification of suitable MRI parameters is ongoing.

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T045

SKIN DELIVERY OF FERULIC ACID FROM DIFFERENT LIPID VESICULAR SYSTEMS

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The aim of the present research is to evaluate the skin delivery capabilities of different lipid vesicular systems, including conventional liposomes (CL), Tween 80-based deformable liposomes (DL), invasomes (INS) and ethosomes bearing ferulic acid (FA) being an antioxidant exhibiting a wide range of therapeutic effects against various diseases. All of the test formulations were characterized for particle size distribution, ζ-potential, vesicular shape and surface morphology, in vitro human skin permeation and skin deposition. Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) defined that all of liposomal vesicles were almost spherical, displaying unilamellar structures with low polydispersity (PDI<0.2) and nanometric size range (z-average no more than 150nm). In addition, all the vesicular systems except conventional liposomes were negatively charged to a certain extent. In vitro skin permeation and skin deposition experiments demonstrated that the permeation profile of ferulic acid through human stratum corneum epidermis membrane (SCE) and the drug deposition in skin were both improved significantly using these vesicular liposomal systems. Permeation and skin deposition enhancing effect was highlighted by the ethosomal system containing 18.0 mg/ml of ferulic acid with an significantly ($P<0.01$) enhanced skin flux (267.8 ± 16.77 $\mu g/cm^2/h$) and skin drug deposition ($51.67\pm1.94 \mu g/cm^2$), which was 75 times and 7.3 times higher than those of ferulic acid from saturated PBS (pH 7.4) solution, respectively. This study demonstrated that ethosomes are promising vesicular carriers for delivering ferulic acid into or across the skin.

T046

CHARACTERIZATION OF SEMISOLID SLN-DISPERSIONS BASED ON PHOSPHOLIPON 90H

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The aim of the present study was the preparation and physico-chemical characterization of semisolid nanoparticular dispersions based on hard fat and Phospholipon® 90 H (P90H), a completely hydrogenated phospholipid. The lecithin-hard fat mixtures - hereinafter referred to as lipid matrices (LM) - served as basis for the production of semi-solid nanoparticular systems. The considered formulations contained - in addition to the lipid matrix (with 10% P90H) - a non-ionic emulsifier for the stabilization (Macrogol-15-Hydroxystearate, Solutol® HS15), thimerosal as a preservative and water as external phase. The lipid matrix content was 10, 12.5 and 15 %, respectively. The systems were prepared by hot pressure homogenization using different homogenizers (EmulsiFlex-C5 from Avestin and Panda from Niro Soavi). The "Avestin systems" were collected in a single vial, the "Niro systems" were filled into several vials due to a bigger sample volume. The partical size distribution for all SLN dispersions was almost monomodal, the z-averages of the dispersions were below 500 nm as far as systems with P90H contents of 10% were concerned. Over a storage period of 6 weeks at room temperature, the dispersions remained stable in relation to their particle sizes and distributions. The use of different homogenizers did not affect particle size and particle size distribution either. Using oscillation measurements, viscous and elastic properties of the formulations could be determined. In addition, consistencies and product stability of the systems could be classified. The linear viscoelastic area, storage and loss modulus, phase angle and the complex viscosity are important parameters to characterize the rheological properties of a given system. The storage and the way of manufacturing the systems seemed to have a significant influence on the rheological properties. One week after production the "Avestin systems" offered a higher consistency and stability compared to the corresponding "Niro systems". Over a storage period of six weeks a decrease in consistency of the systems was generally observed. Furthermore the Niro systems dominated the Avestin systems in terms of consistencies and stabilities that were related to the kind of filling and sampling. Taking a sample at different time points from the same vial had a critical influence on the sample's microstructure.

T047

INVESTIGATION OF THE MECHANISM OF EMULSION STABILIZATION WITH A TRITERPENE EXTRACT FROM THE OUTER BARK OF BIRCH

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A w/o emulsion system stabilized by a triterpene dry extract from the outer bark of birch (TE) was the subject of the present study.
The primary aim was the characterisation of the TE's influence on the interfacial tension between the lipophilic phase and the aqueous phase. Furthermore, oil solubility of the TE and its distribution in emulsions were investigated.
Water-in-oil emulsions were prepared by adding water to a lipophilic phase that consisted of TE finely dispersed in oil by means of an Ultra-Turrax®. Surface tension was measured using the axisymmetric drop shape analysis (ADSA) method. The structure of TE stabilized emulsions was investigated with a confocal Raman microscope (CRM). The oil solubility of the main constituents of the TE was quantified by gas-chromatography.
As a result, it was found that the TE was completely soluble in the oil phase at concentrations < 2.3 mg/ml. Close to its saturation concentration, the TE reduced the interfacial tension between the oil and water by only 5 mN/m.
The total amount of dissolved triterpenes in the oil could be further increased when the added amount of TE exceeded the saturation limit. The supernatant of TE suspensions containing 60 mg/ml revealed a total concentration of triterpenes of 3.3 mg/ml and an interfacial tension of 18.8 mN/m. However, it could be demonstrated, that this supernatant does not form stable o/w emulsions.
Obviously, the presence of suspended TE particles is a necessary prerequisite for the emulsion stabilizing effect of the TE. Accordingly, CRM showed that TE particles covered the water droplets and additionally formed a network in the lipophilic phase.
Thus we conclude from these results, that TE stabilized emulsions are Pickering emulsions. The stability is mainly given by the adsorption of solid particles to the oil-water interface. The surface activity of dissolved triterpenes plays only a minor role.

T048

INVESTIGATION OF THE STABILITY OF O/W PICKERING EMULSIONS STABILIZED WITH COATED AND UNCOATED CALCIUM CARBONATE

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Solid stabilized emulsions (Pickering emulsions) have several advantages compared to classical emulsions. They are surfactant free and thus are attractive for dermal and oral use where classical emulsifiers are sometimes not well tolerated. The aim of this study was to evaluate the suitability of uncoated $CaCO_3$ nanoparticles and their mixtures with stearic acid coated $CaCO_3$ for an o/w emulsion stabilization. Moreover, the effect of particle concentration on emulsion stability was investigated.
Wettability of CaCO3 nanoparticles was characterized by a modified Wilhelmy plate method measuring the advancing contact angles with water.
$CaCO_3$ stabilized Pickering emulsions consisted of $CaCO_3$/ MCT/water (5% / 20% / 75%). The $CaCO_3$ was either pure uncoated $CaCO_3$ or mixtures consisting of 3 parts of uncoated and 1 part of coated particles (mixture 3+1), and equal amounts of coated and uncoated particles (mixture 1+1), respectively.
The effect of the particle concentration was investigated with emulsions containing 3, 4, 5 or 6 g $CaCO_3$.
Physical stability of emulsions was characterized by droplet size measurement on emulsion samples stored for 4 weeks at 23 °C and 40 °C.
It was shown that the contact angle of particulate emulsifiers with water can be used to characterize their polarity. The wettability of particle mixtures was shown to be additive and increases with the amount of hydrophobically coated particles. Stability tests of the emulsions clearly indicate a synergistic effect of particle mixtures. Compared to the emulsion stabilized with pure uncoated $CaCO_3$ emulsions stabilized with the mixtures do not show substantial coalescence or at least reach a stable plateau value within the first days of storage. Furthermore, their terminal droplet size is smaller. This indicates that the particle mixtures are able to stabilize a larger interfacial area than the singular uncoated particles. It could also be demonstrated that increasing amounts of particulate emulsifiers lead to smaller droplet sizes and avoid the risk of droplet coalescence. Thus it can be concluded that stable Pickering emulsions can be formulated successfully using optimized mixtures of hydrophilic and hydrophobic nanoparticles.

T049

FORMULATION, PHYSICAL STABILITY AND CRYSTALLINE STATUS OF POLYHYDROXY SURFACTANT BASED SLN AND NLC

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A pre-requisite to apply lipid nanoparticles on sensitive or irritated skin is to use non-ionic surfactants at low concentrations. They should be low irritant to the skin (often refered as „skin friendly") and should not contain ethylene oxyde groups. Polyhydroxy surfactants may fullfill these requirements. At present no data are available for the use of these stabilizers for lipid nanoparticles (SLN and NLC). A number of different surfactants from the mentioned group were evaluated in the preformulation study. Their wetting ability onto the lipid matrix of nanoparticles were assessed by contact angle measurements to identify suitable candidates for further investigations. From this, polyglycerol 6-distearate (PD) and alkyl (C_8-C_{14}) polyglucoside (AG) were identified as the most suitable candidates. SLN and NLC were produced by hot high-pressure homogenization and analysed in regard to physical stability by dynamic light scattering. The solid state of the particles was assessed by differential scanning calorimetry. In all experiments, the total amount of lipid was kept constant (10% (w/w)). Both surfactants in the conc. of 1% (w/w) led to almost same nanoparticles with a mean diameter between 150 and 200 nm and polydispersity index below 0.15. Measured zeta potential (in distilled water) for both SLN and NLC were higher than |−30mV|. All systems were physically stable over the investigated period of 3 month. AG based SLN had a lower crystallinity index and melting enthalpy than PD based one. NLC stabilized with PD showed a melting event, whereas NLC stabilized with AG did not. The results indicate that polyhydroxy surfactants: a) interact with the lipid matrix and b) can either prevent or accelerate the crystallisation. A possible explanation is that long saturated alkyl chains of PD contribute to the overal solid lipid content inside SLN and NLC, whereas short alkyl chains of AG prevent crystallization.
In conclusion: Polyhydroxy surfactants are suitable stabilizers for both SLN and NLC. However, depending on the molecular structure they interact differently with the lipid matrix of the particles and thus may change their structure. This observation will be a subject of further investigations.

T050

COMPOUNDING OF SUSPENSION-TYPE OINTMENTS WITH DIFFERENT HOMOGENIZERS - A COMPARATIVE STUDY

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Suspension-type ointments were compounded with two commercially available mixing systems and with three roll mills. The systems were compared with regard to the quality of the manufactured ointments.
Suspension-type ointments with 10% salicylic acid and the bases macrogol ointment, wool fat ointment, and white ointment were compounded either with the Cito-Unguator®-e and TOPITEC® mixing systems or with three roll mills (Exakt 50, 50EC, 80E) of different gap size. The manufacturing process was evaluated with regard to the time required for ointment preparation and ointment temperature. The quality of the ointments was examined with respect to homogeneity, particle size distribution, rheological properties and stability.
The homogeneity of the ointments was best with the three roll mills and the TOPITEC® mixing system. Particle size distribution was also best with the three roll mills leading to a particle size between 10 and 40 µm using the smallest gap size. The Cito-Unguator®-e and the TOPITEC® systems led to broader particle size distributions and particles were larger. The rheological properties differed significantly between the ointments prepared with the three roll mills and the two mixing systems. With three roll mills a soft consistency of the ointments accompanied by a low flow limit was obtained, whereas with ointments prepared with the two mixing systems ointment consistency was hard and flow limits were high. This phenomenon may be explained by the significant temperature increase of the ointments during manufacture with the two mixing systems. Time required for preparation of the ointments was not significantly more with the three roll mills. However, some product loss has to be expected.
With three roll mills a better quality of suspension-type ointments may be obtained in comparison to the Cito-Unguator®-e and the TOPITEC® mixing systems: Particle size is smaller, particle size distribution is narrow, ointment stability is better, the temperature of the processed ointments does not increase significantly and the rheological properties are most favorable. Moreover, preparation of ointments with three roll mills allows quality control measures during the manufacturing process before transfer of the ointments into jars or tubes.

T051

FILM FORMING SEMISOLID FORMULATIONS FOR DERMAL APPLICATION

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Generally, formulations for dermal application can be divided into two groups: liquid/semisolid formulations such as creams and ointments and adhesive patches. Whilst semisolid formulations can be applied to large areas of the (diseased) skin, retardation of drug release is more easily achieved by adhesive patches. As some diseases require formulations that exhibit prolonged drug release and can be applied to large areas, a film forming semisolid formulation for dermal application was developed and investigated. The formulations under investigation consisted of an O/W emulsion, containing a thickener, Eudragit NE 30D and/or Eudragit RS 30D as film forming polymers. The influence of different thickeners (HPMC, PVP and PVA) on the stability of Eudragit dispersions was investigated. Free films were prepared from emulsions containing varying compositions of Eudragit NE and RS 30D. The influence of Eudragit composition on the mechanical properties (adhesion and elongation) and the water resistance of free films was investigated. High adhesion, elongation of ≥ 30 % and high water resistance were required. It was found, that the addition of HPMC or PVP to Eudragit NE 30D leads to sedimentation of the Eudragit nanoparticles. PVA did not decrease the stability of Eudragit dispersions and was therefore used for emulsion preparation. Elongation was found to increase with increasing Eudragit NE 30D fraction. Adhesion to glass was expected to increase with increasing Eudragit RS 30D fraction. Interestingly, films containing either Eudragit NE 30D or Eudragit RS30D showed equally low adhesion whilst films containing mixtures of Eudragit NE 30D and Eudragit RS 30D showed an even and high force of adhesion to glass. Adhesion to polycarbonate was found to be generally higher than to glass. As expected, the adhesion to polycarbonate did increase with increasing amounts of NE up to 40 parts NE. At higher amounts of NE, films were destroyed during the measurement because adhesion to the polycarbonate surface was higher than cohesion of the films. Water resistance was found to be enhanced by increasing amounts of Eudragit RS 30D. Desired properties were obtained from formulations containing 60-100 parts of RS.

T052

REDUCED TRANSDERMAL PERMEATION OF TRETINOIN BY NANOENCAPSULATION.

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The aim of this work was to encapsulate tretinoin (TTN) in poly-(epsilon)-caprolactone nanocapsules (TTN-nc) to reduce the topical absorption of the drug and avoid its important side effects.
Four formulations were assayed: A free TTN-solution, a TTN-nc suspension (0.5%) and two hydrogels. TTN-nc suspensions were prepared by the interfacial deposition method developed by Fessi, H. et al (1988)[1]. Hydrogels (0.5%) were prepared by dispersing 0.5 % Ultrez Carbopol ® either in the TTN-nc suspensions or in the TTN-solution. TTN diffusion has been evaluated by means of static Franz diffusion cells. The four formulations were applied by infinite dosing into the donor compartment. The acceptor compartment was filled with EtOH:PBS (pH 7.4) (50:50, V/V) solution. Human heat separated *Epidermis* was used as diffusion membrane. Sampling was performed every 2 h for 32 h. At least five replicates were assayed for each condition.
Formulating TTN in a gel (K_p=0.7±0.1*10^{-6} cm/s), reduced significantly TTN diffusion compared to the solution (K_p=1.8±0.2*10^{-6} cm/s). Encapsulated TTN showed, no difference when applied in form of solution or gel (K_p=0.3±0.1*10^{-6} cm/s), but showed a significantly lower permeation compared to the free drug solution.
TTN transdermal diffusion can be strongly reduced by nanoencapsulation of drug independently of the topical formulation selected as vehicle for the nanocapsules.

1. Fessi, H., Puisieux, F., Devissaguet, J.P., 1988. Procede de préparation dês systèmes coloïdaux d'une substance sous forme de nanocapsules. European Patent 0274961 A1.

T053

COLLOIDAL CARRIER SYSTEMS FOR ENHANCED CUTANEOUS DELIVERY OF HYDROPHILIC DRUGS

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Background The long term treatment of severe Psoriasis with classical systemic therapeutics such as Ciclosporin is accompanied by a high risk of serious side-effects, due to the high dosages necessary to reach the target organ, the skin[1].
Objective The aim of the presented work is to develop a drug delivery system for a potent drug which can be applied topically instead of the oral therapy with its serious side-effects. Thus, a higher concentration at the pharmacological relevant location, the skin can be achieved. Another advantage is the lower exposure for the organism. Consequently, the selected drug needs to be surveyed for a possible dermal application.
Methods First, the physicochemical properties of the substance were determined. The saturation solubility in different lipophilic and hydrophilic mediums was investigated using the method of precipitation. According to Lützhoff et al.[2] the partion coefficients between octanol and buffer with varying pH was evaluated. Further, for quantification we optimized a HPLC method. The isoelectric point (IEP) was also established with the aid of capillary electrophoresis (CE) using UV detection.
Results *Solubility of the substance*: In lipophilic mediums, like Tegosoft DEC®, IPM® or IPP® and in hydrophilic mediums, like water the model-substance was "practically insoluble"[3]. By adding Transcutol P to the lipophilic mediums the solubility could be increased to "very slightly soluble"[3]. In contrast, in water the solubility could be increased to the same range by addition of Propylenglycol or even better by Pentylenglycol. PH values between 5 and 9 improve the solubility behavior of the solution as well. The IEP was evaluated to approx. 3.3.
Conclusion The used model-substance does not possess the optimal properties and requirements for a dermal application. The drug is hydrophilic, practically insoluble and it has also a low permeability[4]. Therefore, it is necessary to develop a colloidal vehicle system, which has the ability to increase the bioavailability of the substance within the skin. Further investigations such as skin penetration of the selected drug incorporated in a colloidal vehicle system have to be performed.

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T054

ALKYLPOLYGLYCOSIDE STABILIZED NLC: INFLUENCE OF SURFACTANT CONCENTRATION ON PARTICLE SIZE & STABILITY

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Introduction: Alkylpolyglycosides (APGs) have been developed by Emile Fischer in the early 1900s. However, large scale production and thus commercial recognition only started about 20 years ago. Thus APGs are often referred to be novel. APGs are nonionic surfactants with interesting properties. They are produced from renewable resources only and are fully biodegradable. After dermal application they are extremely skin friendly and nonirritant. Therefore they are also candidates for the production of skin friendly nanostructured lipid carriers.
Experimental Methods: In this study two APGs with different alkyl chain lengths (C8-C16 and C8-C10 fatty alcohol glucosides, Plantacare 818 and 810 respectively) were used. Nanostructered Lipid Carrier (NLC) (10% lipid phase) were produced by hot high pressure homogenization with surfactant concentrations of 2, 4, 6 and 8% (w/w) respectively. The particle size was investigated by Photon Correlation Spectroscopy (PCS) and Laser Diffractometry (LD). The zeta potential was measured in distilled water with a conductivity of 50 µS/cm and pH 5.8
Results and discussion: The influence of the surfactant concentration on the particle size, surface charge and physical stability of NLC was investigated. All samples produced led to small sizes and narrow particle size distributions (PI < 0.2) during a period of 3 months at room temperature, indicating that APGs possess a very effective stabilization mechanism for NLC. The smallest particles (Plantacare 818 <100nm) were achieved with surfactant concentrations of 6%. Zeta potentials increased from 2%- 6%. More surfactant did not yield a higher zeta potential, indicating that 6% fully cover the surface of the particles.
Conclusion: APGs are suitable stabilizers for the production of skin friendly and physically stable NLCs. APGs follow the rule that smallest particles are obtained if the ratio of surfactant to lipid is equal to 0.6. Therefore, NLC formulations with 10% lipid phase will obtain the best results with 6% surfactant.

T055

ASSESSMENT OF EMULSIFYING PROPERTIES OF ALGAL EXTRACTS

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Semisolid preparations are very important dosage forms to deliver drugs to the skin or to offer nutritional attributes.
They are often composed of a hydrophilic and a hydrophobic phase and, therefore, need to be stabilized by surface-active substances. For this matter low molecular surfactants can be used which often show adverse side effects, like irritation of the skin, allergic reactions or an unwanted penetration-enhancement of substances that can damage the skin [1].
Other substances with higher molecular weight - without the above mentioned drawbacks - can stabilize emulsions by forming a film at the phase boundary layer and by increasing the viscosity of the coherent phase. Known polymeric emulsifiers are for example hydrophobically derivatized carbomers or substituted cellulose ethers.
In this work, algal extracts as renewable primary products were analysed for their potential emulsifying properties that have the previously described advantages and do not need further processing. The examined products were freeze-dried aqueous extracts from different algae (*Saccharina latissima, Ulva lactuca, Palmaria palmata, Undaria pinnatifida, Ascophyllum nodosum*).
The extracts were dissolved in water, mixed with a range of lipophilic phases and homogenized by Ultra-Turrax®. Homogenization parameters and composition of the emulsions were varied and their influence on the stability was determined. HPMC-stabilized emulsions as described by [2] were prepared for comparative purposes. The stability of the resulting emulsions was determined by dynamic light backscattering, microscopy, centrifugation and thermal stress tests.
The pure algal extracts at the used solid contents were not able to stabilize the emulsions. However, it was possible to produce stable semisolid formulations when the viscosity of the coherent phase was increased by the addition of polyacrylic acid, while the negative controls (use of polyacrylic acid alone) led to rapid phase separation.

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T056

FINITE DOSE SKIN PENETRATION EXPERIMENTS IN VITRO: THE ROLE OF THE LATERAL COMPARTMENT

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Franz diffusion cell penetration experiments are widely used to study in vitro skin absorption of topically applied substances. For the detailed examination of full-thickness skin, tape stripping of the stratum corneum (SC) and cryosectioning of the deeper skin layers (DSL) is typically applied[1] and the amount of drug in each compartment is determined.
An important issue especially when performing finite dose experiments is the drug amount in the "lateral" part of the skin. This part of the skin is not in direct contact with the donor solution during incubation and is typically discarded when performing infinite dose diffusion experiments.
However, for finite dose studies a substance flux into the lateral part may lead to considerable difficulties in interpreting and modeling finite dose substance flux due to a rapid depletion of diffusant in the donor compartment.
In this study the role of the lateral compartment and the impact of the experimental methodology[1] (skin stretching before tape stripping and the usage of a smaller punch to separate the deeper skin layers) was examined with the help of computational pharmacokinetic compartment models. The time-dependent flux between the different compartments of the skin (SC, DSL and lateral part) was assumed to follow first-order kinetics. The resulting set of ordinary differential equations was integrated numerically and fitted to experimental data using a multi-dimensional non-linear least-squares and weighted least-squares approach.
The model showed that due to the experimental setup, scaling of the measured masses inside the different compartments is crucial to avoid the creation of "pseudo-lateral" parts that might falsify the total mass recovery results. Furthermore, there is evidence for a non-negligible lateral flux beyond the boundary of the incubated area that should be taken into account when studying and modeling in vitro finite dose dermal absorption.

1 Heike Wagner, N. Z., Claus-Michael Lehr, Ulrich Schäfer. in *Cell Culture Models of Biological Barriers* (ed Claus-Michael Lehr) Ch. 17, 289-309 (Taylor & Francis, 2002).

T057

INVESTIGATION OF THE INHALED FRACTION AND PARTICLE SIZE DISTRIBUTION OF A NEBULIZED ORPHAN DRUG

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AP301 is a synthetic peptide which consists of 17 amino acids and corresponds to a structural motif of the human Tumour Necrosis Factor alpha. AP301 peptide activates lung fluid clearance and protects both endothelial and epithelial lung tissue from microbial toxin- and reactive oxygen species-induced hyper-permeability. It is being developed by Apeptico in various pulmonary indications, such as treatment of the pulmonary oedema in Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS), prevention and treatment of Ischemia Reperfusion Injury during lung transplantation and prevention and treatment of hyper-permeability upon microbial and/or viral infection of the lower respiratory tract and has received Orphan Drug Designation both in Europe and USA.
In order to apply this orphan drug to the lungs, its solution is nebulized using an active membrane nebulizer (Aeroneb Solo, Aerogen Ltd., Galway, Ireland).
The aim of experiments was to investigate the feasibility of inhalational administration of this drug to the lungs. Therefore, it was aimed to gather information about the particle size distribution of the produced aerosol and the output rate of AP301. Three different concentrations (1, 5, 25 mg/ mL) of protein were nebulized. Each concentration was measured three times.
To determine the inhalable fraction of the produced aerosol, a modified Pari Compas breath simulator (Pari GmbH, Starnberg) set-up with 5 different stages was used. After each run, the different parts were washed out with a water-methanol-mixture and the amount of API for each fraction was determined via UV spectroscopy.
As a next step, information about particle size distribution of the produced aerosol was obtained using a precooled Next Generation Pharmaceutical Impactor (NGI, Copley Scientific, Nottingham, UK) at a flow rate of 15 L/min. The residue of each stage was again analysed via UV spectroscopy.
In addition, the gained results were compared to those of laser diffraction testing (Sympatec HELOS Inhaler module, Sympatec GmbH, Clausthal-Zellerfeld).
Regarding the fine particle fraction and inhaled fraction, the results show a good feasibility of nebulization for an aqueous solution of AP301.

T058

SYSTEMIC DELIVERY OF LYOPHILIZED PROTEINS VIA INHALATION

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Since proteins and peptides are poorly absorbed in the gastrointestinal tract, the lungs are a promising target for administering these types of substances because of their large inner surface area, thin epithelium, and relatively low protease activity [1]. Insulin as model protein was lyophilized with L-glutamic acid and polysorbate 80 as stabilizers forming low-density, stable but easily dispersible cakes. An active inhalation device, based on dispersion by means of air impact, was developed warranting fine dispersion of cakes suitable for lung delivery. Aerosol characteristics of lyophilisates were determined using in-vitro cascade impaction (Next Generation Pharmaceutical Impactor, NGI).
In this study, device parameters dispersing pressure and dispersing time were evaluated regarding their influence on resulting fine particle fraction (FPF), which is a measure for the mass of API deposited in deeper lung regions [2]. A design of experiment was set up, varying dispersion pressure between 1 and 3 at and dispersion time between 0.1 and 1 s. Results, calculated with Design-Expert® 8 software, show a significant influence of dispersing time, even though it is a weak one. The resulting FPF values fluctuate around 30%, wherein the highest values are achieved with minimum parameter settings. The delivered doses found in the NGI decrease with increasing parameter settings, whereas the vial and device retention are relatively stable. The study has shown that direct aerosolization of lyophilized proteins is possible and has some potential for systemic delivery of drug products. Future research will target an elevation of both fine particle fraction and delivered dose.

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T059

DEVELOPMENT OF A DRY POWDER NASAL VACCINE FORMULATION

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Mucosal vaccination via the nasal route is a promising alternative to the systemic application of vaccines as the nasal tissue is well equipped with immunocompetent cells and is easily accessible. In contrast to soluble antigens, particulate antigens do not only elicit a local but also a systemic immune response [1]. Hence a particulate dry powder formulation comprising good stability is favorable.
In this study, primary particles containing BSA (bovine serum albumin) as model antigen and chitosan as mucoadhesive and stabilizing excipient were produced by spray-drying (SD) and powder blends were prepared using 2, 5 and 10% of the spray-dried microparticles and four different coarse carriers: microcrystalline cellulose, trehalose, crystalline and spray-dried mannitol (fraction 45-90 µm). Particle size distribution, uniformity of delivered dose, stability of the protein and nasal deposition of the powder mixtures were determined. For the in-vitro deposition experiments a nasal cast model [2] which is based on a computer tomography scan of a human nose, and a nasal dry powder device (PowderJet) were used. Deposition studies of the pure microparticles showed that about 40% of the protein was not deposited in the nasal cavity because the particles were too small (~ 3 µm). The particle size of the formulation should be in the range of 10 to 100 µm in order to avoid inhalation. Accordingly, the inhalable fraction was significantly reduced to about 5% by the introduction of powder mixtures. In this case, the microparticles are co-deposited with the coarser carrier in the nasal cavity. In contrast to the pure microparticles the powder mixtures could also be readily dispersed by means of the PowderJet and showed an excellent uniformity of the delivered dose. In conclusion, the preparation of interactive powder blends was found to be an easy and effective way of delivering SD-stabilized antigens to the nasal cavity.

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T060

NOVEL DRUG-CARRIERS FOR PULMONARY ADMINISTRATION UTILIZING A TEMPLATE-ASSISTED APPROACH

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Treating pulmonary diseases today requires the frequent administration of drugs. Currently no system for prolonged release is available, which results in a weak adherence to medication for chronic pulmonary diseases.
Recent scientific publications indicate a high importance of shape concerning the internalization of particles by alveolar macrophages. Surprisingly, the local morphology at the point of contact with the phagocyte is profoundly dominating the fate of the particle and size is only of relevance, if the volume exceeds the capacity of the macrophage. The possible geometries in order to prevent ingestion are numerous and include cylindrical forms such as tubes, rods and fibers.
Learning from the hazardous consequences of asbestos exposition, it was discovered that microfibers easily reach the deep lung and cannot be cleared. Therefore the geometry of a fiber is ideal shape for a progressive pulmonary drug-carrier system.
Utilizing these finding we are aiming at the preparation of cylinders that can not be readily phagocytosed. We selected a template-assisted approach to from the cylindrical shapes in the confined space of a membrane. The sacrificial membrane is subsequently dissolved in order to liberate the cylinders.

T061

IN VITRO INVESTIGATIONS FOR MAGNETIC LUNG DRUG TARGETING: INFLUENCE OF AEROSOL FLOW VELOCITY ON THE DEFLECTION IN MAGNETIC FIELDS

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For magnetic drug targeting to the lung a suspension of magnetic nanoparticles in water (ferrofluid) containing cytostatic drug is atomized and the magnetizable aerosol droplets are inhaled. A magnetic field is arranged close to the lung in the region of lung cancer. The aerosol droplets are deposited in the diseased lung due to the magnetic field. It may be possible to achieve therapeutic drug concentrations at the tumor site.
The ferrofluid was atomized by two different nebulizers (eFlow and Pari Boy) in order to vary the median mass diameter of the droplets which was determined by laser diffraction. The deflection of magnetic droplets was investigated in the magnetic field of two opposing circular disc permanent magnets (r = 25 mm, l = 15 mm) with a magnetic remanence of 1450 mT at a distance of 20 or 40 mm between the magnetic poles and in an electromagnetic field (r = 22 mm, I = 1 A, 3 A and 5 A) with a distance of 40 mm between the two opposing north poles. About 50 mg iron were sprayed into a square tube (5 x 2 cm) which was placed centrally between the two opposing magnets. The walls to the magnets were covered with paper. A reproducible aerosol flow velocity was generated by using a vacuum pump. We investigated the degree of aerosol deposition at 4 and 8 l/min. The paper with the deposited nanoparticles was decomposed and the iron content was quantified by flame atomic absorption spectrometry.
The eFlow generated aerosols with a droplet size of 4 to 6 µm and the Pari Boy of about 3 µm. The size decreased with increasing ferrofluid concentration. The strongest magnetic field gradients were generated at the edges of the opposing circular disc magnets. The gradients near the pole surface were up to 120 T/m. At a distance of 2 cm between the two opposing magnets a nearly homogeneous gradient of 15 to 20 T/m was achieved. The gradients decreased with increasing distance. Atomic absorption spectrometry showed that up to 63% of iron were intercepted onto the paper. The best deposition was achieved by eFlow. This was caused by the large droplet size of up to 5.16 µm that is generated by the vibrating membrane. Large droplets contain more magnetite nanoparticles and are deflected more easily. But this high deposition with large aerosol droplets is not applicable for magnetic drug targeting. Aerosol droplets of such a size only reach the upper airways and lead to adverse reactions due to high deposition in mouth and trachea.

T062

COMPARISON OF POROSITY FROM GRANULES AND SLUGS MADE BY DRY GRANULATION

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Porosity is regarded as an important factor for the compactability of granules, produced either by wet or dry granulation. For granules obtained from roller compaction, in many cases the porosity is estimated by measuring the ribbon.

This work shows first results of comparing mercury porosimetry data from granules and the slugs from which they are gained by milling. Therefore slugs of plain Microcrystalline Cellulose (MCC) as well as binary mixtures from MCC with Calcium Phosphate (Di-Cafos PA) and MCC with Eudragit RS PO were produced by briquetting.
The briquetting was accomplished by a Flexitab® (Röltgen, Solingen), a pneumo-hydraulic-single punch press using 10 mm biplan punches.
10 tablets of each batch were reserved for measuring the porosity, the remaining tablets were milled by a KitchenAid® with grain mill Attachment to produce the granules.

The porosity was measured with a Pascal 140/440- System with Win-Pascal1.05 Software (Thermo Fisher Scientifics, Waltham, USA). The data of the granules were treated as already published at APV-Congress on Malta, March 2010.

The results indicate that for slugs of plain Avicel with a high porosity the difference to the granule porosity is high, too. By decreasing slug porosity with higher compaction pressure, results get closer to the values for the granules. This behaviour is changing for the powder blends. According to the compaction behaviour of the powder the difference in porosity decreases with a higher fragmentation tendency.

Referring to those results it seems to be incorrect using data from slug measurements for describing granule porosity.

T064

THE USE OF HYDROPHILIC RELEASE MODIFIERS FOR SOLID LIPID EXTRUSION

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Solid lipid extrusion is a technique which makes it possible to process a wide range of excipients and APIs together with solid fats to extrudates of defined properties. By variation of the solid fat type or more effectively by adding a hydrophilic release modifier it is possible to change the release behaviour. Polyethylene glycol (PEG) as an example has been used in preliminary studies as such a hydrophilic agent. Beside PEGs further excipients are available to enhance the dissolution from solid lipid extrudates. The aim of this study was to compare the effectiveness of different groups of release enhancers, having different chemical and physical properties. Extrudates consisting of 55 % diprophylline and 45 % tristearin (reference extrudate) and 50% diprophylline, 45 % tristearin and 5 % release enhancer were manufactured. Conventional super disintegrants (Kollidon® CL and CL-SF, Ac-Di-Sol®, Primojel® and L-HPC) made the first group of release enhancers. Among the various disintegrant types differences could be detected. Ac-Di-Sol® and Primojel® had the strongest influence on the release rate reaching 80 % released drug after less than 30 minutes compared to the reference extrudate, which took 300 minutes to reach the same amount of released drug. The influence of Kollidon® CL and CL-SF was quite different. The CL-type enhanced the release rate much stronger than the CL-SF-type since its mean particle size is higher than that of the CL-SF type. The second group of excipients consisted of hydrocolloids (Tylose® H 20 and H 30000 and Metolose® 65 SH 50 and 4000), which form gel structures. Hydrocolloids of high viscosity levels led to higher release rates than those of lower viscosity levels. Readily water soluble excipients made the third group of release modifiers (Polyglykol® 10000, sodium chloride and mannitol) and very probably leave pores in the lipid matrix during dissolution out of which the API can be released. Polyglykol® 10000 played an extraordinary role since its release enhancing effect was the strongest. Sodium chloride and mannitol instead did not influence the release rate as PEG did. The extrudates were physically characterized by differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). In summary this study showed that it is easily possible to manufacture extrudates with different release characteristics by integrating an appropriate release modifier to the matrix.

T065

HOT-MELT EXTRUSION - A NEW PRODUCTION TECHNIQUE FOR ORAL APPLICABLE FILMS?

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In the last few years, mucoadhesive oral films have become important in the individual therapy of children as well as adults. Hot-Melt extrusion (HME) is a common technology used to produce films in the plastics industry. Since HME has been established in pharmaceutics for many years, this technology was a natural choice for use in film formation.

This study dealt with the screening of different polymer substances in production of mucoadhesive films. A Coperion ZSK18MC and a BBA CompactContiCooler 40/40 were used to produce endless films of about 25mm width and about 200µm thickness. For each formulation, the extrusion parameters (screw speed, barrel temperatures, feed rate) as well as the roller cooler parameters (roll temperatures, speed) were optimized in order to obtain a film of adequate shape and size. In a new approach, the strand was plastically deformed into a thin film and cooled at the same time.

The sugar alcohols (Mannitol, Xylitol) were investigated based on their high chemical stability. Both substances result in a brittle extrudate which could not be plastically deformed into thin films. This was attributed to the rapid crystallization. The sugar ethers Hydroxypropylmethylcellulose and Hydroxypropylcellulose degrade during the extrusion. Polyethylene glycol (PEG) of different molecular weights (10k - 4000k) showed an excellent extrusion behavior. While the low molecular weight PEG were quite soft, the high molecular weight was hard and sturdy.
Soluplus had an excellent film formation behavior, however brittle films were obtained. A combination of 10% PEG 20k and Soluplus resulted in films of adequate mechanical properties.

In conclusion, a film formulation was found which could be processed with HME.

T066

HOT MELT EXTRUSION OF ISOMALT AS HYDROPHILIC CARRIER FOR POORLY SOLUBLE SUBSTANCES

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Hot melt extrusion is a process of rising interest to increase the dissolution, the solubility and the bioavailability of poorly soluble drugs [Lit.1]. Typically, hydrophilic, water-soluble polymers are used to produce solid solutions or solid dispersions of the drug. But there are also some interesting approaches to use sugar alcohols, such as sorbitol, mannitol or isomalt, as a carrier for such poorly soluble substances. In this case, the hydrophilic carrier increases the wettability of the drug and may enhance its dissolution. Isomalt is a disaccharide alcohol which is composed of two stereoisomers: 1-O--D-glucopyranosyl-D-mannitol dihydrate (1,1-GPM dihydrate) and 6-O--D-glucopyranosyl- D-sorbitol (1,6-GPS).
In this study, the influence of different extrusion temperature programs on the morphology of the extrudates was investigated. For a rapid dissolution, it is necessary to develop the process to obtain an amorphous extrudate which shows acceptable long term stability. As a surrogate for the stability, the re-crystallisation behaviour was investigated with Differential Scanning Calorimetry (DSC) and X-ray Powder Diffractometry (XRPD). Additionally the influence of the storage conditions of the extrudate on the re-crystalisation behaviour was investigated. To investigate the influence of the isomalt quality on the stability of the extrudate in the amorphous form, two different isomalt grades were used: galenIQ^{TM} 720 with an equimolecular amount of both stereoisomers and galenIQ^{TM} 721 with a stereoisomer ratio 3:1 (GPS:GPM).

The results show, that the temperature program of the extrusion process as well as the isomalt grade influences the glass transition temperature (T_g) of the extrudate. Also the re-crystallisation is strongly influenced.

Acknowledgements: Thanks to Beneo-Palatinit for the kind donation of galenIQ^{TM} and to Gabler GmbH for allocating an extruder for these experiments.

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T067

DEVELOPMENT OF LOZENGES BASED ON EXTRUDED STARCH

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During the past four decades there has been intensive research on modified- and controlled-release drug delivery systems. The most common way to produce controlled-release tablets is by applying wet granulation or direct compression techniques. Both occasion high costs. Hot-melt extrusion shows many benefits compared to ordinary manufacturing techniques. The continuous mode of operation and the potential to save production costs are the main advantages.
The aim of this work is the development of lozenges for buccal use.
An important aspect was to characterize the suitability of different starches for extrusion. Extrudates made of five different types of starch (corn starch, Eurylon®5, pea starch, potato starch and Waxilys®200) were produced by using a Brabender single screw extruder (type 811201) fitted with a slit die (length: 3 mm, width: 8 mm,. height: 2,5 mm), A cork borer (diameter: 8 mm) was used for shaping biplanar lozenges.
Three different batches were compared: Placebo- menthol- and clove oil-lozenges. All samples were stored in a climatic exposure test cabinet (climatic zone 2 according to ICH Q1F Guideline). Glass transition temperatures were determined by differential scanning calorimetry. Water contents of the extrudates were measured by thermogravimetry. It could be shown, that an increase of moisture content leads to a decrease of glass transition temperature. For investigating the hardness of the extrudates a tablet hardness distribution tester was used. Hardness of the extrudates rapidly increased during the first two weeks of storage time, then remained static. The degree of hardness could be linked to the type of starch used for extrusion. Crystallinity of the extrudates was determined by x-ray diffractometry. According to the x-ray data, for nearly all samples extrusion led to a complete amorphisation of the starch. Scanning electron microscopy proofed the results of the x-ray diffractometry. Organoleptic features of the lozenges were evaluated by questionnaires. Sufficient dissolving times in mouth of more than 30 minutes could be observed. Drug release rates could be related to the ratio of amylopectin and amylose of the used starch. Waxylis®200 showed strongest cooling intensities, whereas lowest cooling intensities were obtained for Eurylon®5.

T068

NIR-CHEMICAL IMAGING FOR THE EVALUATION OF DRUG DISTRIBUTION IN SOLID MATRICES

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Near infrared chemical imaging (NIR-CI) is a versatile analytical technique that combines conventional imaging and spectroscopy. Spatial and spectral information is simultaneously obtained. This rapid and non-destructive method is employed to investigate the distribution of ingredients in pharmaceutical products.
Aim of this study was to investigate the suitability of NIR-CI for evaluating the distribution in solid matrices like melt extrudates. The extrudates contained 20 % metoprolol tartrate (Microsin, Bucharest) as active ingredient (API), mannitol (Pearlitol® 160C, Roquette, Lestrem) and poloxamer 188 (Lutrol® F 68, BASF, Ludwigshafen) as excipients. The matrices are delivered by a solid dosage pen, a novel dosage system for personalised medicine, which cuts the extrudates into tablet-like slices for individual dosing[a]. The homogeneity of the extrudates and the uniformity of the individual parts were evaluated with NIR-CI (NIR-CI 2450, Malvern Instruments, Worcestershire). Spectra (1200-2400 nm) of the API and the excipients were recorded and compared with the spectra of the native extrudates or slices containing individual doses. Multivariate data analysis was employed to investigate the distribution of the API and the excipients.
Although calculation and visualization of the distribution was possible, the interpretation of the data however is difficult. In comparison with the reference spectra of the pure substances, a spectrum of an extrudate area with a calculated high concentration is a hybrid of all three ingredients. The differences between the spectra of calculated high and low concentrations are very small, indicating a homogenous distribution of the ingredient.
For the evaluation of slices´ uniformity, more than 900 spectra of an individual slice were averaged and compared to the mean spectra of different extrudate parts. The mean spectra were very similar indicating the uniformity of the slices. This could be confirmed by determination of content via UV-spectroscopy.
In conclusion NIR-CI is suitable for the evaluation of the dose uniformity, but for the distribution of individual ingredients, data should be carfully evaluated.

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T069

STARCH-BASED PELLETS PREPARED BY HOT MELT EXTRUSION AND DIE-FACE PELLETIZATION

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Aim of the work was to develop a solid solution or dispersion of drugs with different starches via hot melt extrusion which is directly pelletized with a die face micropelletizer. The influence of the different starches, drugs and additives on the extrusion process, the physicochemical properties and the release profile of the resulting pellets was investigated. The extrudates were produced on a twin screw extruder (ZSE 18 HPPH 40D, Leistritz; Germany) fitted with varying dies. The strand was immediately pelletized using a die face Micropelletizer (LM P18 PH, Leistritz; Germany).
Different drugs (Ibuprofen, Paracetamol, Phenazon and Tramadol-HCl), additives and starches were mixed with a tumble blender and filled into the gravimetric dosing unit (K-PH-CL-24-KT20, K-Tron; Switzerland) of the twin screw extruder. The powder was fed into the extruder simultaneously mixed with water and extruded using temperatures between gelatinisation temperature and 100 °C. With scanning electron microscopy the morphology of the resulting pellets was examined. The cristallinity of the pellets was analyzed by X-ray diffractometry. Phenazon and Tramadol*HCl form a solid solution whereas Paracetamol and Ibuprofen can only be dispersed in the starch-matrix with the used extrusion parameters. The gas pycnometric density of the pellets was determined using a helium pycnometer (Ultrapycnometer 1000T, Quantachrome; Germany). The apparent density was measured with a mercury porosimeter (Pascal 140, Thermo Fisher Scientific Inc.; US). Specific surface area analysis was performed using Nova 3000-Series BET, (Quantachrome Corporation; Germany).
Particle size distributions were measured by laser diffraction (Laserdiffractometer HELOS, Sympatec GmbH; Germany). Dissolution studies show that the release profile is conditioned by the used starch, additive and active ingredient. Mathematical modelling of the drug release data indicates that the drug release is mainly based on swelling and diffusion and can be altered by the additives and active ingredients.
According to the available data, die face pelletization of hot melt extruded starch is an interesting innovative way to produce drug containing pellets with nearly monodisperse particle size distribution, very low porosity and surface area, the desired shape and release characteristics.

T070

ENTERIC COATING OF PELLETS PREPARED BY POWDER VS. SOLUTION LAYERING TECHNIQUE USING FLUID BED EQUIPMENT

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Drug loaded pellets are usually prepared by solution or suspension layering technique (SL) when using fluid bed processor. Former investigations have proved that drug powder layered (PL) pellets can successfully be prepared using a modified fluid bed processor Ventilus 25 (Innojet Herbert Hüttlin, Germany), too. In this study pellets prepared by both layering techniques were subsequently coated with an enteric polymer and products were compared regarding coating efficiency, coating level and drug release profile. Additionally, the coating results for three PL batches are compared to each other to show reproducibility.
All pellets were prepared with the same water soluble model drug using either SL or PL technique. Eudragit L 30 D - 55 (Evonik Röhm GmbH, Germany) was chosen as enteric coating polymer and applied using the Ventilus 2.5. The dissolution of the coated dosage forms was measured corresponding to Ph Eur. When the enteric coating layer is thick enough no drug substance is released at a pH value below 5.5. In these experiments pellets were coated with a preferably thin functional film to quantify and compare required coating levels. When the applied coating level is barely sufficient to form a continuous film, small differences in coating thickness lead to differing drug release profiles. Therefore drug release profiles were compared when drug release was 6 - 10 % after 120 min.
PL pellets provided a better process performance because pellets were less electrostatic. Furthermore PL pellets could be as efficiently coated as SL pellets and coating efficiency was reproducible for the three PL pellet batches.
Drug release profiles of the three coated PL batches were equal. As soon as the enteric layer was disrupted PL pellets released the drug moderately faster than SL pellets. This effect can be explained by their larger surface area due to cavities within the drug layer and may be advantageous to improve the solubility of poorly soluble drugs.
At this point of process development PL pellets require a significantly higher coating level to result in a drug release profile comparable to SL pellets. Therefore further investigations focus on the improvement of surface area properties of PL pellets by adapting the process parameters.

T071

COLON TARGETING: THE APPLICATION OF ENTERIC COATINGS TO PROTECT CHITOSAN COATED TABLETS IN THE GIT

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Colon targeting refers to the specific release of oral medications in the colon. It is suitable for peptide and protein drugs which may be absorbed in the colon, but have to be protected against low pH in the stomach and enzymes in the small intestine. Furthermore, colon targeting is also important for the local therapy of colonic diseases, e.g. ulcerative colitis or colon cancer. To achieve this goal, solid dosage forms containing the drug are coated with a polymer film, which is designed to be specifically degraded in the colon by the enzymes present in its microflora.

In our studies the polymer chitosan, which is known to be enzymatically degradable, was used. Tablets were coated in the Innnojet Air Coater 025 fluidized bed apparatus (Innojet Technologies, Steinen, Germany). Different types and film thicknesses of chitosan were chosen to investigate their influence on drug release. Dissolution studies were carried out using an USP type I method (Paddle method, 100 rpm, 37 °C). The simulated intestinal fluid (SIF) was a phosphate buffer (pH 6.8) representing the conditions in the small intestine and the physiological large intestine. Because of the solubility of chitosan in the stomach, different additional enteric coatings were investigated to protect the chitosan film coating. Disintegration studies with and without an additional enteric coating were carried out as described in Ph. Eur. for enteric coatings in HCl (0.1 N) at 37 °C and compared to each other.

It could be shown that the drug release of chitosan-coated tablets could be reduced to below 16.0 % for the first 180 minutes, presenting the early drug release in the small intestine. Afterwards, the film coatings were intact for over 20 hours under physiological conditions. The type of chitosan did not influence the drug release. The disintegration time of chitosan-coated tablets increases with higher amounts of applied chitosan in 0.1 N HCl. Enteric coatings can inhibit tablet disintegration for more than 120 minutes. Afterwards the drug release in pH 6.8 is similar to tablets without enteric coating or even reduced. These characteristics would be beneficial in the possible application of chitosan coated tablets for colon targeting. Enzymatic degradation studies with these batches and conditions are under progress.

T072

DEVELOPMENT AND CHARACTERISATION OF LIPID SHELL ANTHOCYANIN MICROCAPSULES

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Anthocyanins are secondary plant ingredients which are responsible for the red colour of fruits e.g. in bilberrys (Vaccinum Myrtillus L.). Amongst others they are used in the prevention of colon cancer due to their antioxidative capacity [1]. The aim of this work was to microencapsulate these polyphenolic compounds to protect them against early degradation. Because of their intended use as a dietary supplement we selected only excipients which are accepted as food ingredients.
For the characterisation of the particles ESEM and light microscopic pictures were taken. For the ESEM pictures the particles were treated with a razor blade to reveal their inner structure. It is evident that the particles have more than one core. In comparison with the light microscopic pictures the existence of multi cores is approved. The dark red zones are cores filled with the acidic extract solution. ESEM analysis shows a broad particle size distribution. These results were confirmed by laser diffraction. Mean Particle diameter (d_{50}) was about 177 µm (n=4). The use of an Ultra-Turrax® for particle preparation causes a decrease of the incorporation rate and did not result in a narrower particle size distribution.
The release of the incorporated anthocyanins was tested using UV-spectroscopic pH-differential method [2]. During 120 minutes incubation in SGF (without enzyme) only 12 % of the incorporated anthocyanins were released. In phosphate buffer pH 6.8 release was faster. During 120 minutes 22 % of the incorporated anthocyanins were released. Stability tests showed that the Particles are stable over a period of at least four weeks at room temperature (in the dark). Particle size distribution did not change during the observation period. Also the flowability remains constant.
It was shown, that stable microparticles were developed. A lipid shell coated hydrophilic cores. Release studies showed, that the capsules are able to save the incorporated anthocyanins from early degradation.
The authors would like to thank the DFG (MA 1648/6-1) and the DFG/AiF-Cluster “Bioaktive Inhaltsstoffe aus mikrostrukturierten Multikapselsystemen” for financial support.

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T073

ELECTROSTATIC CHARGING IN A FEED FRAME OF A TABLET PRESS

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Electrostatic charging may be a problem in the process of tableting especially when high speed rotary tablet presses are used. Powder flow through the hopper can be hindered or tablets can stick together or to parts of the tablet press. The electrostatic charge occurs when particles come into contact and are separated afterwards. This can be the case for the powder flow in the hopper, the feeder, the dies or during movement of the tablets on the turret or other parts of the tablet press.

In this study the influence of material and operating conditions of a feed frame with paddles on the electrostatic charging of pharmaceutically used powders was evaluated. A mixer consisting of a cylindrical bowl and a paddle mixer was used as a model for the feeder. The electrostatic potential was measured by an electrostatic sensor which was positioned above the mixing bowl.

The influence of the material of the mixing vessel and the paddle as well as the rotating speed of the stirrer was investigated in a 2^3 full factorial design with 2 replicates under controlled environmental conditions. Paracetamol was chosen as model drug, because it is known for its ability to electrostatic charging. The material of the stirrer (metal or plastic) showed a significant influence on the electrostatic charge whereas the material of the mixer bowl and the rotating speed had no significant influence. The measured differences of the electrostatic potential were in the order of several kV.

In a second experimental set other powders and powder mixtures used in tableting were evaluated. The conducting and grounded metal stirrer was superior over the insulating glass or plastic stirrer in the model. For the construction of feeders in tablet presses the usage of insulated feed frame paddles should be avoided and all machine parts should be well grounded.

T074

QUANTIFICATION OF STICKING TO THE PUNCH SURFACES DURING TABLET MANUFACTURE – A COMPARATIVE STUDY

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Sticking is a common problem observed during tablet manufacture. In the present study material adhering to both, the upper punch and the lower punch, is quantified using HPLC. Ibuprofen is chosen as model drug because of its high tendency of sticking to punch surfaces. The content of ibuprofen in the formulations is varied from 10 % to 90 % (w/w) using either Ludipress® or Microcelac® 100 as direct compression excipient. Tableting is performed with an instrumented rotary die tablet press equipped with flat-faced punches of 10 mm diameter. The compaction forces are 7, 10 and 13 kN, respectively. Following each compaction run the upper punch is removed from the machine and the punch surface is cleaned from ibuprofen using 5 ml of methanol for the quantification via HPLC. The amount of residual powder from the lower punch is determined with a swab method using a Q-tip soaked with methanol. Subsequently, the Q-tip is immersed in 5 ml of methanol. Quantification is carried out after an extraction period of at least 24 h.
Sticking to the upper punch as well as to the lower punch is decreased with increasing compaction force. Higher compaction forces increase the cohesivity within the tablet. Furthermore, electrostatic interactions between the chrome-plated punch surface and the powder particles are reduced leading to reduced adhesion forces. Sticking to the upper punch is observed with all formulations. However, the amount adhering to the punch surface with the Microcelac® formulation containing 10 % of ibuprofen is found to be below the detection limit of the HPLC method. Sticking of ibuprofen to the lower punch surface could neither be observed with the Microcelac® formulations containing up to 20 % ibuprofen nor with the Ludipress® formulation with 10 % drug content. However, compaction of tablets with higher drug contents lead to a residue in the center of the lower punch. The punch is completely covered with residue at drug contents of 50 % with both, the Microcelac® and the Ludipress® formulation. Furthermore, the amount of ibuprofen adhering to the upper punch is considerably higher than that sticking to the lower punch. The extent of sticking is assumed to be affected by the mechanism of detachment of the punches from the tablet: The upper punch is separated from the tablet in vertical direction. In contrast, the tablet detachment from the lower punch occurs by tangential shear forces induced by the take-off bar.

T075

ORALLY DISINTEGRATING MINI-TABLETS WITH HYDROCHLOROTHIAZIDE - A NOVEL DOSAGE FORM FOR PAEDIATRIC USE

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Orally disintegrating mini-tablets (ODMTs) are a new concept for paediatric drug delivery. These tablets with a diameter smaller than 3 mm are intended to disintegrate in the oral cavity in less than 30 seconds. This novel dosage form is particularly suitable for younger children, infants and toddlers, and could be manufactured with conventional tabletting facilities.
The aim of this study was to investigate the feasibility of various ready-to-use excipients for direct compression of stable and child-appropriate ODMTs with hydrochlorothiazide.

Ludiflash® (BASF, Germany), Parteck® ODT (Merck, Germany), Pearlitol® Flash (Roquette, France), Pharmaburst® 500 (SPI Pharma, USA) and Prosolv® ODT were used as directly compressible tableting excipients. Sodium stearyl fumarate (Pruv®, JRS Pharma, Germany) was used as lubricant and hydrochlorothiazide from Unichem (India) served as model-drug.
Biconvex mini-tablets with 2 mm diameter were directly compressed with a rotary tablet press (IMA Kilian, Germany) by using a 19-tip mini-tableting tool (Ritter, Germany). Varying compression forces were applied.
Crushing strength (Texture Analyzer, Stable Micro Systems, UK), friability and the time required for complete wetting of an ODMT (simulated wetting test-time, SWT), as well as uniformity of dosage units were determined.
All formulations compressed with 5kN and 8kN indicated a sufficient friability below 1%. The crushing strength of these ODMTs ranged between 2.64N and 17.6N. These major differences depend on varieties in compactability of the excipients. The ODMTs showed SWT-times between 3.1s and 25.2s. Further "uniformity of dosage units" was demonstrated, according to Ph.Eur. monograph 2.9.40.

All excipients are suitable for preparation of ODMTs with hydrochlorothiazide. Orally disintegrating mini-tablets are supposed to be very useful formulations for the treatment of young children and may be considered as a new technology platform for paediatrics.

T076

ENHANCEMENT OF GRISEOFULVIN RELEASE FROM LIQUISOLID COMPACTS AND OPTIMIZATION THEREOF

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The potential of liquisolid systems to improve the release of poorly soluble drugs was investigated using griseofulvin as model drug. The in vitro release rates of this drug, formulated as directly compressed tablets with crystalline griseofulvin and as liquisolid compacts, respectively, were studied.

The new formulation technique of liquisolid compacts was used to convert liquid drug medications into acceptably flowing and compactable powders. Several liquisolid tablet formulations were prepared using a mathematical model to calculate the appropriate quantities of powder and liquid ingredients.
Therefore, solutions and suspensions of griseofulvin in the non-volatile liquid vehicle PEG 300 were mixed with selected powder excipients. In the present study the powder excipients typically used for the liquisolid technique (Avicel® PH200 and Aerosil® 200 in a ratio of 20:1) were compared to Neusilin® US2, an amorphous magnesium aluminometasilicate with an extremely high specific surface area.

The liquisolid formulation containing a drug solution showed a significantly higher drug release rate than the directly compressed formulation containing micronized griseofulvin. This may be explained by the fact that the drug in the liquisolid system is already dissolved.
Moreover, it has been shown that with compacts containing drug suspensions the release rate increases with decreasing amounts of undissolved drug in the liquid vehicle. However, a decrease of suspended drug in the liquid vehicle at a given drug amount requires a higher volume of liquid. Because the powder excipients can only adsorb limited amounts of liquid while maintaining good flow and tableting properties this results in an increase in tablet weight.
Neusilin® US2 with its extremely high liquid adsorption capacity allows the production of liquisolid formulations with lower tablet weights than formulations with Avicel® PH200 and Aerosil® 200.

T077

DEVELOPMENT OF A GASTRO-RETENTIVE EXTENDED RELEASE FORMULATION OF FUROSEMIDE

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Furosemide is a very potent loop diuretic. The clinical application is restricted to emergency medication due to its rapid elimination. Extended release formulations fail due to the fact that absorption is limited to the upper small intestine. To overcome this problem a gastro-retentive formulation is designed. The prolonged residence in the stomach is accomplished by intense swelling of the tablet matrix. Erosion is prevented by means of a highly flexible protective coating. The drug release is controlled solely by diffusion.
Varying matrices and pore forming excipients were tested to achieve a swelling independent of pH and ionic strength of the dissolution media. When replacing the gelling agent CMC-Na against agar, the matrix showed a more viscous behavior. The dissolution rate of the BCS class IV drug was accelerated by means of coprecipitation with PVP K-25 from methanolic solution.
Drug release was tested in a standard paddle apparatus (900 mL, 37 °C, 50 rpm) in media with pH 5.8, 4.5 and 1.7 to simulate the varying conditions of the stomach in the fasted and fed state. Furthermore, the impact of mechanical forces on the drug dissolution was investigated using a novel dissolution stress test apparatus. The applied testing regime allows the generation of pressure waves at predetermined intervals to simulate gastric motility in the pylorus region in the fasted state.
The swelling of uncoated matrices was nearly unaffected by pH. Water influx was susceptible to the plasticizer used. Drug release was also dependent on the kind of pore former employed. Dissolution profiles applying the standard test were nearly linear. In the case of the dissolution stress test an increase in dissolution rate was observed directly after applying the pressure waves. However, the stress equivalent to maximum motility in the fasted stomach did not result in a complete burst of the dosage form.
Unfortunately, the drug release decreased markedly with increasing acidity of the dissolution media. This was most likely due to the restricted solubility of furosemide in the applied media. To overcome this issue, the integration of buffer substances into the tablet matrix will be examined in further studies.
Financial support granted by the German federal ministry of education and research (FKZ: 03IP612) is gratefully acknowledged.

T079

QUALITY CONTROL OF ALUMINIUM BLISTER FOILS: QUANTITATIVE ANALYSIS OF HEAT SEAL LACQUERS

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According to GMP Guidelines the quality control of the pharmaceutical industries comprises not only “Inprocess controls” and controls of bulk and drug products but also the control of all starting materials. In addition to analysis of active substances and excipients, the control of packaging materials is obligatory. A compound of many packages used for drug products, especially blisters are aluminium foils. To achieve a sealability of the aluminium material against polymer foils, e.g. the formable foils of blister packaging, the aluminium usually is coated with a 5 to 9 g/m² layer of heat seal lacquer. As the heat-sealed joint is the most critical compound of blisters related to mechanical stability and impermeability against outside influences it must be assured, that the heat seal lacquer is of reproducible quality. Therefore it is analyzed concerning identity, firmness of sealing joint and grammage (mass per unit area). Currently the generally applied method for determination of grammage is a gravimetric method which requires a removal of the lacquer. Using this method as reference, several instrumental methods for determination of the heat seal lacquer grammage were developed and compared to each other concerning parameters required for validation of analytical procedures in the ICH guidelines Q2(R1). Interferometric, IRRAS and beta backscatter techniques were well suitable for the measurements. Using these techniques novel procedures applicable for routine quality control of pharmaceutical packaging materials are suggested.

T080

EXPRESSION ANALYSIS OF DRUG TRANSPORTER PROTEINS IN EXCISED HUMAN NASAL MUCOSA AND CELL LINE RPMI 2650

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The nasal mucosa is an interesting site for systemic application of drugs, since it has a number of advantages as a delivery route for drugs with systemic action, including easy accessibility, extensive vascular supply and avoidance of first-pass-metabolism. In the development of drugs for nasal application, identification and characterisation of different efflux and uptake systems are necessary. P-glycoprotein (P-gp) and multidrug resistance-associated proteins (MRP) are classified as ATP-binding cassette (ABC) transporters based on their sequences, organisation of the ATP-binding domains and efflux function. ABC proteins represent a large family of integral membrane transporters that utilize the energy of ATP hydrolysis to carry specific substrates across membranes. The solute-carrier gene (SLC) superfamily encodes another large family of membrane-bound transporters, located in almost every cellular and organelle membranes. Proteins of the SLC family include passive transporters, ion-coupled transporters and exchangers.
A comparison was performed between excised human nasal mucosa from turbinectomy surgeries and our in-vitro model based on immortalised human nasal epithelial cells (RPMI 2650) concerning the mRNA expression of different efflux and uptake transporter proteins. To profile the mRNA expression of eight ATP-binding cassette transporter (P-gp, MRP 1 till 5, BCRP and CFTR), two oligopeptide transporter (PEPT1 and 2) as well as four organic cation transporter (OCT 1 and 3, OCTN1 and 2) the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was applied. All bands for the DNA amplification products of selected ABC and SLC proteins (except for OCT3) were detected in both samples and located at positions with the expected sizes. However, a difference in signal strength was observed. First permeation studies using specific P-gp substrate Rhodamine 123 in the presence and absence of verapamil as inhibitor of this transporter were performed in both apical to basolateral (ab) as well as basolateral to apical (ba) directions with the in-vitro model. No significant difference in the permeation coefficients of Rhodamine-123 between ab and ba direction was detected and no influence of verapamil could be observed, suggesting the absence of P-glycoprotein in the RPMI 2650 model.

T081

EXAMINING THE SUITABILITY OF RIBOFLAVIN/UVA-TREATMENT FOR TISSUE ENGINEERING APPLICATIONS

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To improve the mechanical stability of a tissue engineered human cornea construct, which is used as an in vitro model for drug absorption studies, the collagen matrix of this construct is to be strengthened by collagen cross-linking. A suitable method to induce photooxidative cross-linking of collagen fibrils is UVA irradiation combined with riboflavin as a photosensitizer.
After riboflavin/UVA-treatment, the viscoelastic properties of the collagen matrix and the molecular weight of its proteins, as well as cell viability of the human corneal keratocytes (HCK) incorporated in the stromal matrix, were analyzed depending on the dose of irradiation using oscillation rheology, gel electrophoresis and the MTT assay, respectively. In addition, the cell damage to the HCKs after riboflavin/UVA-treatment was also analyzed in monolayer cultures. Various luminescent cell assays were performed to clarify whether the decrease in cell viability was a consequence of apoptosis or necrosis. Furthermore, fluorescent double staining was carried out using an apoptotic/necrotic cells detection kit.
The improvement of mechanical properties was low, whereas resultant cell damage was considerable and dose-dependent. When lower doses of irradiation were used, the reduction of cell viability was triggered by apoptosis while necrosis supervened for increased doses of irradiation.
We conclude that in contrast to clinical applications, the riboflavin/UVA-treatment does not seem to be a suitable method to obtain a sufficiently firm stromal matrix including vital keratocytes to build a tissue engineered human cornea construct to be used as an in vitro model for drug absorption studies.

T082

CHARACTERISATION OF A CORNEA CONSTRUCT FOR DRUG ABSORPTION STUDIES AND COMPARISON WITH EXCISED TISSUE

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The increased use of ophthalmic products over the past decades has lead to an enlarged demand of transcorneal drug absorption studies in vitro and in vivo. Due to the lack of alternatives animal experiments are widely-used for such studies as well as ocular cytotoxicity tests. Models of the human cornea based on tissue engineering could avoid several disadvantages of animal experiments such as ethical concerns and poor standardisation. Despite these numerous disadvantages so far no available in vitro model is generally accepted. In the context of prevalidating a human cornea construct (HCC) for in vitro drug absorption experiments, this study describes the analysis of its barrier characteristics and compares it with that of excised rabbit and porcine cornea.
Human cornea constructs were cultivated under serum free conditions on permeable polycarbonate filters (Transwells®, Costar) using SV 40 immortalised human keratocytes (HCK-Ca) and immortalised human epithelial cells (HCE-T). Briefly, using Keratinocyte Growth Medium (KGM, Lonza, USA) and an advanced cultivation schedule the HCC showed a suitable barrier. Its equivalence to native tissue was analysed by comparative absorption experiments with isolated rabbit and porcine cornea. For this purpose model substances with a wide range of molecule characteristics were used, including hydrophilic dye sodium fluorescein, lipophilic dye rhodamine B, macromolecule FITC labeled dextran (FD-4), β-blocker timolol, steroid hormone dexamethasone, prostaglandin analogue bimatoprost and antiviral drug aciclovir. Diffusion experiments with HCCs were performed in Transwells® according to a standardised protocol, while permeation studies with excised corneas were accomplished in the vertical Ussing chamber system (Havard Apparatus). To investigate the intra-laboratory repeatability the construct cultivation as well as the permeation studies were performed independently by different experimenters.
Reconstructed human cornea constructs exhibited a barrier in the same range as excised corneas. Resulting from the standardised cultivation procedure HCCs showed high reproducibility and lower standard deviation than excised tissue. Therefore the HCC could turn out to be a promising in vitro alternative to the use of ex vivo tissue.

T083

INLUENCE OF INLB 321 CD ON IMMORTALIZED HUMAN DERMAL KERATINOCYTES AND INTACT ORGANOTYPIC CO-CULTURE

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Internalin B (InlB) is an invasion protein of Listeria which facilitates its uptake into the host cells via activation of the receptor tyrosine kinase c-Met. It was proposed that receptor dimerization, which is essential for activation, is mediated through an InlB dimer. The variant InlB 321 CD[1] (crystal dimer), i. e. a dimerized fragment of Internalin B, was designed to stabilize the InlB dimer in solution and study the effects on c-Met activation. In previous studies, recombinant InlB 321 CD produced in E. coli was used in binding studies and in in vitro scatter assays[1]. It was shown, that InlB 321 CD is a stronger agonist than both monomeric InlB 321 and Internalin B. In human skin the c-Met receptor is mainly expressed on keratinocytes where it leads among other effects to proliferation. Therefore, the present project aims at the influence of dimeric as well as monomeric InlB 321 on re-epithelialization of dermis with keratinocytes. Subsequent to studying the potency of InlB 321 CD on the HaCaT cell line (human dermal immortalized keratinocytes), the effects of InlB 321 CD on bioengineered skin of a keratinocyte-fibroblast co-culture as a human skin equivalent were studied to test whether the morphology of the bioengineered skin as a model of intact skin was affected.
Methods: A viability assay via MTT was carried out on HaCaT monolayer, after incubation with InlB 321 CD in a range of 0.01 nM – 0.5 nM; 30 nM HGF (a physiological c-Met receptor agonist) served as positive control. Intact organotypic co-cultures were determined in the same manner using MTT test. The histological analysis of morphology was performed of treated and untreated constructs by polymer-embedding and haematoxylin and eosin staining.
Results: Within a range from 0.05 nM to 0.5 nM InlB 321 CD showed a significant increase in viability of HaCaT monolayers compared to medium and monomeric InlB 321 of the equivalent concentrations whereas neither morphology nor viability of intact organotypic co-cultures were affected.

[1]Ferraris, D.M. et al., Ligand-Mediated Dimerization of the Met Receptor Tyrosine Kinase by the Bacterial Invasion Protein InlB, J. Mol. Biol. (2009), doi:10.1016/j.jmb.2009.10.074

T084

mRNA EXPRESSION OF METABOLIC ENZYMES IN HUMAN CORNEA, CORNEAL CELL LINES AND CORNEA CONSTRUCT

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Drugs from ophthalmic formulations are mainly absorbed into the eye via corneal route. However, few is known about drug metabolism during transcorneal passage. The objective of this study was to determine the mRNA expression of phase I and II isoenzymes in human corneal tissue, corneal cell lines as well as human cornea construct as in vitro model for drug absorption studies.
The polymerase chain reaction was used to profile the mRNA expression of 10 cytochrome P450 enzymes and 7 phase II enzymes in three human corneal cell lines HCE-T (epithelial), HCK-Ca (stromal) and HENC (endothelial cells) as well as in tissue engineered cornea equivalent. Furthermore, the human colon adenocarcinoma cell line Caco-2, human corneal epithelium obtained from donor corneas and human liver tissue were investigated.
The immortalized human corneal epithelial cell line (HCE-T) showed marginal mRNA expression of the P450 enzymes 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5. Signals for CYP1A2 and CYP2B6 were absent. The tested phase II enzymes GSTA4-4, GSTO1-1, GSTP1-1, NAT1, NAT2, SULT1A1 and UGT1A1 could be detected in HCE-T. In the case of phase II enzymes higher expression levels of mRNA were observed in comparison to CYP 450 enzymes. The mRNA expression in the immortalized human corneal keratocytes cell line (HCK-Ca) was quite similar to HCE-T. However, the P450 enzymes 2A6, 2C9 and 3A5 as well as the phase II enzyme NAT2 were not discovered. The human corneal endothelial cell line (HENC) showed the same mRNA pattern like HCK-Ca cells. PCR for the cornea construct shows similar result as obtained for the cell lines HCE-T and HCK-Ca, whereas CYP2C19 and NAT2 were not detected.
Overall, the mRNA expression of tested phase I and phase II enzymes in the three corneal cell lines and the cornea equivalent correlated well with the expression pattern found in human cornea epithelium in vivo.

T085

GENE EXPRESSION OF NOGGIN AND CHORDIN IN PRE-OSTEOBLASTS IN RESPONSE TO BMP-2

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Non healing bone defects can be successfully treated with high dosages of Bone morphogenetic protein 2 (BMP-2) to recruit and differentiate stem cells as has been shown with Infuse™. But therapies involving potent growth factors also bear a risk for severe side effects. BMP-2 is a highly regulated osteogenic protein. There are internal antagonists for BMP-2, e.g. noggin and chordin. A gene silencing strategy for these internal BMP-2 antagonists may allow for a reduction of effective BMP-2 dosing. The aims of this study are: 1) to determine gene expression of noggin and chordin in rat bone marrow derived mesenchymal stem cells (rMSC) and human adipose tissue derived stem cells (hADSC) during osteogenic supplementation with and without BMP-2; 2) to show siRNA transfection of both cell types with a control siRNA and 3) to investigate the effect of siRNA against both BMP-2 antagonists. Results: Osteogenic differentiation protocols for both hADSCs and rMSCs were applied successfully. Expression profiles of noggin and chordin were examined by total RNA-extraction with Trizol and reversed phase PCR / gel electorphoresis / GelRed staining. rMSCs showed reliable noggin expression already during the first days of osteogenic differentiation with and without supplementation with BMP-2. Chordin mRNA, on the other hand, was expressed in very low amounts and was only detectable in some samples on days 3 to 6. For hADSCs the situation was found to be different. In all hADSC groups chordin was expressed particularly strong between days 5 and 14, while noggin expression was weak. BMP-2 supplementation enhanced noggin expression in hADSCs but the levels found for chordin were not reached. Transfection of rMSCs with fluorescent labelled siRNA using HighPerfect (Qiagen) and Lipofectamine™ RNAiMAX (Invitrogen) showed 97 % fluorescent cells in flow cytometry. Cell death assay, an assay used to prove successful transfection by gene silencing of vital proteins, on the other hand, was only successful for hADSCs, particularly in combination with Lipofectamine™ RNAiMAX. Finally, we examined the effects of siRNA against noggin. First results showed down-regulation of noggin between days 2 and 4.

T086

COMPARISON AND EVALUATION OF ABC TRANSPORTER EXPRESSION IN DIFFERENT CORNEA MODELS AND A CACO2 CELL LINE

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The aim of most drugs for eye treatment is reaching the inner space of the eye indicating that these substances need to pass the cornea. Up to now parameters like transcorneal permeation rate and toxicity of ophthalmic drugs, needed for registration and approval of human medicines, are mostly tested and analyzed on research animals. This led to a great need to replace animal test systems by in vitro cell culture models. In this context the identification and characterisation of the different substance uptake systems in the cornea is of interest. Besides the presence of tight junctions, the permeation through the corneal epithelium could be strongly influenced by the presence of several efflux transporter systems. The expression at mRNA and protein level of ABC transporters in different corneal models was investigated. A comparison between a monolayer of immortalised human corneal epithelial cells submerged and air-liquid-interface cultivated, previously established human cornea construct, excised human and animal cornea, concerning the mRNA expression of efflux transporters was performed using reverse transcriptase PCR. The presence of protein expression was analyzed using Western blot and immunohistochemistry, whereas the level of activity was determined using a bidirectional permeation assay with specific substrates and inhibitors for each transporter. The mRNA expression of nine efflux transporters (MDR1, MRP1-6, CFTR and BCRP) was examined and a similar pattern was obtained between the epithelial corneal cell line, the constructs, human and rabbit cornea. However, mRNA expression of MDR1, MRP4 and CFTR was not detectable in porcine cornea cells. The permeation studies with rhodamine 123 and verapamil showed an absence of MDR1 in all models except in the Caco2 cell line (positive control) and rabbit cornea, where the permeation with [H^3]Erythromycin and MK571 on the other hand showed the expression of MRP2 in the epithelial corneal cell line. The confocal images and Western blot confirmed the previous results.
In conclusion, two discrepancies were pointed out in this study, showing not only a difference in the expression of efflux transporters at mRNA and protein level, but also between the three investigated species.

T087

EVALUATION OF CULTURE CONDITIONS OF TWO HUMAN CORNEAL CELL LINES EMPLOYED FOR THE ESTABLISHMENT OF A NEW CORNEAL MODEL TO ASSAY DRUG PERMEABILITY

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Objective: The main purpose of the joint research project study was to establish an in vitro corneal permeability model using two immortalized cell lines as well as ensure the reproducibility between three different laboratories. Within the joint research project “Prevalidation of an serum free Cornea Construct for Drug Diffusion Studies” the cell culture conditions of the two used cell types were evaluated to set for the three dimensional hemi cornea constructs.

Methods: Seeding density was varied from 450,000 cells to 100,000 cells per flask (75cm^2) for HCK-Ca cells, 450,000 to 300,000 for HCE-T cells. After 7 days the HCK-Ca and HCE-T cell lines were seeded onto membranes of Polycarbonate and with two different pore size (3.0 µm and 0.4µm) and polyester filters (pore size 0.4 µm; Transwell-Clear^{TM}). The TEER was determined and permeability of Fluorescein and Propranolol.

Results: The optimum number of cells (8 mio) harvested from 75 cm^2 flasks after 7 days for HCK-Ca cells than a seeding density of 1.0e5 cells was used; for HCE-T cells (8 mio) 3.0e5. The vitality was always above 90%. Higher densities decreased the number of harvested cell down to 50%. The TEER values varied from 970 to 1560 Ω.cm^2. Monolayer grown on Transwell Clear^{TM} (Polyester, pore size 0.4 µm) has developed higher TEER values compared to polycarbonate Transwells (pore size 3.0 and 0.4 µm). The TEER of cells grown in polycarbonate filters with 0.4 and 3.0 µm pore size were similar. No difference were observed between permeability coefficients (P_{app}, 10^{-6} cm*s^{-1}) for FLU 0.28±0.030 and PRO 13.2±0.58, 11.8±0.55 for polycarbonate membrane. FLU permeability (0.20 cm*s^{-1}) was similar for cells grown on polyester Transwells, the permeability PRO permeability (8.95 cm*s^{-1}) was lower compared to the permeability measured with cells grown on polycarbonate Transwells.

Poster
Pharmazeutische Biologie

B088

ISOLATION AND CHARACTERISATION OF HUMAN ELASTIN

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Elastin is one of the most abundant proteins of the extracellular matrix (ECM). Depending on the anatomical requirements of vital tissue, elastin forms different types of structures with a characteristic organisation of highly cross-linked elastic fibres inside the ECM. Damaged fibres, which may occur as a consequence of processes such as enzyme dysregulation, pathological conditions and aging, result in a loss of elasticity. To understand the structural changes of elastin during these processes, it is necessary to gain insight into the morphological and molecular constitution of the native protein. Such studies may aid in the development of new, adequate therapies to conserve and to reconstitute the elasticity and the functionality of the ECM.
An isolation method of Daamen *et al.* (1) was adapted and the experimental conditions were optimised to obtain highly purified and intact elastin fibres out of the ECM of vital human tissue using single biopsies. The morphological constitution of elastin isolated from skin, aorta and cartilage was investigated by scanning electron microscopy (SEM). Furthermore, elastin was digested with different elastases and the released peptides were analysed by HPLC coupled to tandem mass spectrometry (LC/MS/MS). Through the identification of the cleavage products, the primary structure of different elastins, including the splice variants of tropoelastin and post-translational modifications, was investigated. With this novel experimental approach, it is not only possible to visualise the structure of mature elastic fibres, but also to characterise elastin on the molecular level to allow comparison between different human tissues. Moreover, it is possible to identify potential changes and modifications of elastin, for instance in aged or UV-exposed skin or in tissues affected by diseases such as aneurysm or cancer development. Furthermore, intact mature elastin and its precursor tropoelastin are important substrates to investigate the elastinolytic abilities of proteases (2).

(1) Daamen, W. F. *et al.*, Isolation of intact elastin fibers devoid of microfibrils. *Tissue Eng* 2005, **11,** *7-8*, 1168-1176.
(2) Heinz, A. *et al.*, Degradation of tropoelastin by matrix metalloproteinases. *FEBS J.* 2010, **277 (8)**, 1939-1956.

B089

LYNGBYAZOTHRINS A-D, NOVEL ANTIMICROBIAL AND CYTOTOXIC CYCLIC UNDECAPEPTIDES FROM *LYNGBYA SP.*

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Infectious diseases belong to the most frequent causes of death in the world. Although there are meanwhile more than 90 drugs in clinical use, the therapeutic efficiency is strongly influenced by the increasing number of resistant pathogens. On account of this the search for innovative and more effective compounds has become crucial importance. Cyanobacteria are known as a useful source of bioactive secondary metabolites to fill the pipelines for novel active pharmaceutical leads. Especially the genus *Lyngbya* exhibits a large number of natural metabolites including antibiotic, anticancer, cytotoxic, antifungal and antiviral activities. Among other bioactive products such as pyrroles, amides, alkaloids, lactones, derivatives of fatty acids and cyclic peptides and lipopetides of mixed polyketide synthase/nonribosomal peptide synthetase origin represent the largest part. Here we present four novel cyclic undecapeptides, lyngbyazothrins A (**1**), B (**2**), C (**3**) and D (**4**), which were isolated by bioassay-guided fractionation of a crude methanol/water extract from the cultured, filamentous cyanobacterium *Lyngbya* sp. 36.91 as binary mixtures (**1**/**2** and **3**/**4**). Their structures were elucidated by analysis of 1D and 2D NMR spectra, ESIMSMS, ESITOFMS and amino acid analysis. Three unusual amino acids were present and identified as 4-methoxyhomophenylalanine in **1** and **3**, homophenylalanine in **2** and **4** and 3-amino-2,5,7,8-tetrahydroxy-10-methylundecanoic acid (Aound) in all compounds. Only **3** and **4** had an additional *N*-acetyl-*N*-methyltyrosine unit whose carboxyl group is bound to the 5-hydroxyl group of the Aound residue. The mixture of lyngbyazothrins A (**1**) and B (**2**) showed only low antimicrobial activity against *Micrococcus flavus*, whereas the mixture of lyngbyazothrins C (**3**) and D(**4**) was active against *Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Candida maltosa.* Further investigations of the cytotoxic activity revealed that only the mixture of **3** and **4** as well as purified **3** showed an intense cytotoxic effect against a human urinary bladder cancer cell line, whereas an effect could not be seen for **1** and **2**. First insights in the structure-response relationships by chemical degradation suggest that the acyl residue at C-5 of the Aound unit plays an important role in antimicrobial activity.

B090

CHEMICAL AND BIOLOGICAL INVESTIGATIONS OF MANUKA HONEY

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Manuka honey is mainly gained from New Zealands endemic Myrtaceae *Leptospermum scoparium* J.R. et G.Forst. Its increasing clinical use in wound management originates from its special antimicrobial effects. Recent work identified 1,2-dicarbonyl methylglyoxal (MGO) as a major antibacterial compound [1] which appears in Manuka honey in high levels and is formed from dihydroxyacetone during storage [2]. In this study several Manuka honeys were investigated for antibacterial activity, MGO content and phenolic compounds. Antibacterial testing was done by agar diffusion assay as well as in the epidermis model ´cow udder teat´ [3]. Aqueous dilutions of Manuka honeys were able to inhibit growth of multi-resistant strains of *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa*. In these honeys, MGO amounts ranging from 400 to over 1000 mg/kg were found. However, no hydrogen peroxide was detected in Manuka honeys. In comparison: a rape honey contained only 3 mg/kg MGO but high amounts of hydrogen peroxide. It showed inhibiting effects on both *Staphylococcus* strains. If MGO was added to a non Manuka honey the resulting bacterial inhibition was the same as for a Manuka honey with comparable MGO amount. Detected and quantified phenolic compounds such as phenyllactic acid or methyl syringate did not exert antimicrobial activity on the tested strains. Osmotic effects did not contribute to inhibiting effect. Therefore, it appears likely that clinical benefits of Manuka honeys are proportional to its 1,2-dicarbonyl methylglyoxal content.

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B091

THE INTERACTION BETWEEN THYMOL AND EDTA AGAINST MULTIRESISTANT BACTERIA

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Ethylenediaminetetraacetic acid (EDTA) is a known chelating agent of Ca^{+2} and Mg^{+2} ions. The bi-valent metal ions are responsible of many chemical and biochemical reactions, which promote food destruction. Furthermore their basic role in the protection of bacterial cell wall especially of gram-negative bacteria. EDTA has been used as a food preservative and antimicrobial agent. The interaction between EDTA and several antibiotics against different strains of bacteria including multiresistant strains has been studied and many combinations such as the combination between EDTA and carbenicillin, oxytetracycline and others showed synergistic effects.
Thymol is a monoterpene and major component of the essential oil of *Thymus* species (Lamiaceae). It shows strong antimicrobial properties.
The aim of this study was to investigate the interaction between EDTA and Thymol against multiresistant bacteria. Two methods were performed to evaluate the activity of several combinations against *Methicillin-resistant Staphylococcus aureus* (MRSA ATCC 10442), Vancomycine resistant *Enterococcus* (VRE ATCC 31299) and *Klebsiella pneumonia* (ATCC 700306) namely Checkerboard dilution and time-kill curve methods. Synergism is defined as an FIC index ‹ 0.5 by checkerboard dilution method and as a ≥100-fold or 2-$\log_{10}$ decrease in colony count after 24 h by the combination compared with that by the most active single agent by time- kill curve method.
The results obtained in Checkerboard dilution method indicate additive and indifferent effect against Gram-positive and Gram-negative bacteria with an FIC index between 0.7 and 2. A synergistic effect was recorded only against *Klebsiella pneumonia* as an FIC ‹ 0.5.
EDTA was found to enhance the bactericidal activity of thymol in time-kill curve; strong bactericidal activity was achieved with some combinations, whereas the two substances alone did not show the same degree of bactericidal activity after 24h.

B092

BIPHENYL FORMATION IN FIRE BLIGHT-INFECTED *MALUS DOMESTICA* CULTIVARS

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Fire blight is the most dangerous bacterial disease of the Pyrinae. This Rosaceous subfamily includes economically important fruit trees, such as apple (*Malus domestica*) and pear (*Pyrus communis*). Fire blight is caused by the pathogen *Erwinia amylovora* and can lead to the death of the infested plant. After pathogen attack, the plants produce biphenyls and the structurally related dibenzofurans as phytoalexins. The precursor of these defence compounds, 3,5-dihydroxybiphenyl, results from the condensation of benzoyl-CoA with three molecules of malonyl-CoA, catalyzed by biphenyl synthase (BIS). A series of *Malus* species and *M. domestica* cultivars was investigated after inoculation with *E. amylovora*. In *M. domestica* cv. ‘Holsteiner Cox’, the formation of biphenyls and dibenzofurans was detected in the stem. This cultivar is being used as a model system for studying the regulation and localization of the biosynthesis of biphenyl and dibenzofuran phytoalexins.

B093

BIOCHEMISTRY OF GLUCOSINOLATE HYDROLYSIS: ANALYSIS OF THE INTERACTION BETWEEN MYROSINASE AND SPECIFIER PROTEINS

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Specifier proteins are part of the glucosinolate-myrosinase system present in the Brassicaceae, including nutritionally valuable vegetables and spices (e.g. broccoli and mustard). For the plant, glucosinolates are defense compounds. For humans, they are of note because they may have beneficial health effects. For example, regular consumption of broccoli is associated with a reduced incidence of certain cancers. This effect is mainly attributed to isothiocyanates which are the common products of the myrosinase-catalyzed hydrolysis of glucosinolates upon tissue damage. In the presence of specifier proteins, the hydrolysis is redirected to other products, e.g. nitriles or epithionitriles, and a lower proportion of isothiocyanates is produced. Specifier proteins have no enzymatic activity on glucosinolates, but likely act on the aglyca released by myrosinase. Experiments have shown that if epithospecifier protein (ESP) and myrosinase are separated spatially, isothiocyanates are formed *in vitro*. Thus, it seems that the ESP needs to interact with myrosinase. However, it has also been shown that there is no stable interaction between ESP and myrosinase.

In order to detect the presumably transient interaction between myrosinase and specifier proteins, we used a label transfer method with a trifunctional crosslinker Mts-Atf-Biotin (Pierce). First, the purified myrosinase from *Sinapis alba* was biotin-labeled over the Mts moiety of the linker by disulfide bonds. After an initial incubation with a purified recombinant nitrile-specifier protein (*Arabidopsis thaliana* NSP3) and allylglucosinolate, the Atf moiety was activated by UV light. This allows the transfer of the biotin label to proteins in a distance of 11.1 Å expected only for specific protein-protein-interaction. After the disulfide bonds had been reduced, the label transfer was analyzed by immunoblotting. As a result, we were able to detect the biotin label on NSP3 but not on carbonic anhydrase used as a negative control. Thus, NSP3 and myrosinase interacted in our *in vitro* assay. The next step is to characterize the interaction in more detail and to determine the site of interaction in NSP3 and myrosinase.

B094

EVOLUTION OF SPECIFIER PROTEINS IN THE BRASSICALES

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Glucosinolates are secondary metabolites found predominantly in the order Brassicales including nutritionally valuable vegetables and spices like broccoli, rucola, horseradish, and mustard. Health benefits associated with consumption of these foods are attributed to the isothiocyanates released when glucosinolates are hydrolysed by co-occuring myrosinases upon tissue disruption. For the plants, isothiocyanates are defence compounds toxic to insects, nematodes and microorganisms. Depending on the glucosinolate side-chain structure, the presence of specifier proteins results in the formation of alternative products, e.g. nitriles, epithionitriles and organic thiocyanates, with divergent biological activities. These products appear to lack the positive health effects and to be less effective in direct plant defence. Because of the high variability in the composition of the about 120 known glucosinolates and the varying occurence of specifier proteins with different specificities in different species, but also in different organs and developmental stages of a single plant, we wanted to get a better insight into the evolution of this protein family. Our approach was to identify and characterise specifier proteins from glucosinolate-containing plants from different families of the Brassicales and to subject their amino acid sequences to phylogenetic analyses.

Species that produce non-isothiocyanate products upon glucosinolate hydrolysis were selected based on a phytochemical screening among 28 species belonging to six families. GC-MS analysis of autolysates of seeds, and if available, seedlings, leaves and flowers, showed that non-isothiocyanates products were only formed in 15 species belonging to the Brassicaceae. Based on known amino acid sequences of nine specifier proteins from Arabidopsis, broccoli, and garden cress, we designed degenerate primers to identify cDNAs encoding specifier proteins. After cloning of twelve full-length cDNAs from six additional species, we characterised the corresponding proteins by heterologous expression in *E. coli* and constructed phylogenetic trees using different algorithms.

In conclusion, amino acid sequence similarity does not appear to be a sufficient parameter to predict catalytic activity of specifier proteins. Phylogenetic analyses propose a common ancestor of all specifier proteins and at least two independent origins of thiocyanate-forming activity from epithiospecifier-activity.

B095

CLONING, EXPRESSION AND MODELLING OF P5βR-LIKE ENONE REDUCTASES FROM VARIOUS ANGIOSPERMS

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5β-configured cardenolides are still of great medicinal and economical importance in the treatment of cardiac insuffiency in humans. As far as their biosynthesis is concerned the stereospecific reduction of progesterone to 5β-pregane-3,20-dion is often referred to as one key step, because here the characteristic *cis* configuration between ring A and B is formed. The reaction is catalysed by the enzyme progesterone 5β-reductase (P5βR), which has been cloned from several members of the genus *Digitalis* and functionally expressed in *E. coli* [1]. The crystal structure of *Digitalis lanata* P5βR has been solved revealing a novel class of short chain dehydrogenases/reductases (SDRs), with only two of the typical catalytic residues (K147 and Y179) being conserved [2]. An orthologous gene (VEP1) has also been cloned from the model plant *Arabidopsis thaliana*, which does not produce cardenolides. The respective recombinant enzyme was also capable of reducing progesterone stereo-selectively *in vitro* [3]. Both enzymes were capable of reducing other steroidal, cyclic or non-cyclic enone substrates [4].
In this work we report the cloning of orthologous genes from various cardenolide-containing and cardenolide-free medicinal important angiosperms including *Mentha piperita*, *Gomphocarpus fruticosus*, *Atropa belladonna*, *Plantago major*, *Nerium oleander Erysimum crepidifolium* and *Erysimum rhaeticum*. With the exception of the *A.belladonna* protein we succeded in the heterologous expression in *E. coli.* The functionality was shown using TLC, GC-MS and HPLC. As the proteins share 67 to 96 percent sequence identity on amino acid level with the *D. lanata* 5β-POR we used this structure (*PDB 2v6g*) as a template for modelling. We included the substrate progesterone into the 3D models and compared the binding-sites of the functional active enzymes. In addition to the expected catalytic Y179 and K147 residues another five amino acids could be identified within the substrate-binding pocket, namely W106, G145, F153, M215 and F343 being conserved in all enzymes. The residues seem to cluster around the proximal part of the substrate and may be involved in the positioning of the enone-structure-element. The importance of the structurally conservation of these residues will now be investigated using site-directed mutagenesis.

B096

BENZALDEHYDE DEHYDROGENASE INVOLVED IN BENZOIC ACID FORMATION

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Despite its simple structure, the biosynthesis of benzoic acid is poorly understood. Cell suspension cultures of *Sorbus aucuparia* (Rosaceous subtribe Pyrinae, formerly subfamily Maloideae) respond to elicitor treatment with biphenyl and dibenzofuran accumulation. These two classes of phytoalexins are benzoic acid-derived polyketides. The formation of benzoic acid proceeds *via* benzaldehyde as an intermediate. Benzaldehyde dehydrogenase (BD) catalyzes the last reaction of benzoic acid biosynthesis by converting benzaldehyde to benzoic acid, which is finally activated by a CoA ligase to give benzoyl-CoA. Biphenyl synthase (BIS) then condenses benzoyl-CoA with three molecules of malonyl-CoA, leading to formation of the carbon skeleton of the inducible defence compounds. Detection and characterization of BD in elicitor-treated *S. aucuparia* cell cultures was suggestive of benzoic acid biosynthesis *via* a non-*β*-oxidative pathway. The preferred substrate for BD was benzaldehyde (K_m = 49 µM). Cinnamaldehyde and hydroxybenzaldehydes were relatively poor substrates. BD activity was dependent on the presence of NAD^+ as a cofactor (K_m = 67 µM).
A cDNA encoding aldehyde dehydrogenase (putative BD) was cloned from elicitor-treated cells. The ORF encodes a 54.8 kDa protein. No *N*-terminal targeting signal was identified by analysis of the amino acid sequence. The transcriptional level of the putative *BD* gene was significantly higher than that of the *BIS* gene, which is in agreement with the changes in the specific BD and BIS activities after elicitor treatment. These results provide first insight into benzoic acid metabolism in the economically valuable subtribe Pyrinae.

B097

HYPERFORIN BIOSYNTHESIS: CDNA CLONING OF ISOBUTYROPHENONE SYNTHASE

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Hyperforin is an important antidepressant constituent of Hypericum perforatum (St. John´s wort, Clusiaceae) [1]. Cell cultures of the related species H. calycinum were found to contain the homologue adhyperforin and to a low extent hyperforin, when grown in BDS medium in the dark. Cell-free extracts from the cell cultures contained isobutyrophenone synthase (BUS) activity catalyzing the stepwise condensation of isobutyryl-CoA with three molecules of malonyl-CoA to give phlorisobutyrophenone, the hyperforin skeleton [2]. BUS is likely to be a type III polyketide synthase (PKS). The aim of the present work was cDNA cloning of BUS. H. perforatum plants and H. calycinum cell cultures were used for the isolation and reverse transcription of poly (A+) mRNA pools. Degenerate primers that matched conserved motives of known PKS sequences were designed. PCR with combinations of these primers led to the amplification of a new cDNA fragment that was extended by 3´ and 5´ RACE techniques to give the full-length clone. The resulting sequence shared 88% identity with other PKSs and is a promising candidate to encode BUS. Heterologous expression of the open-reading frame in E. coli for functional analysis is in progress.

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B098

BENZOPHENONE SYNTHASE FROM *HYPERICUM CALYCINUM* CELL CULTURES: CDNA CLONING AND FUNCTIONAL EXPRESSION

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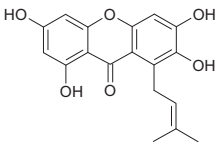
Hypericum is a medicinally important genus (Clusiaceae). *H. perforatum* (St. John's wort) is the best-known member of the genus and widely used as an antidepressant agent. Cell suspension cultures of the related species, *H. calycinum*, form 1,3,6,7-tetrahydroxy-8-prenylxanthone upon elicitation with yeast extract. Xanthones thus appear to serve as phytoalexins in *Hypericum* species, as reported previously. In addition, they exhibit antitumour, anti-HIV, and antimicrobial activities [1].



1,3,6,7-tetrahydroxy-8-prenylxanthone

The carbon skeleton of xanthones is formed by benzophenone synthase (BPS), which catalyses the condensation of benzoyl-CoA and three molecules of malonyl-CoA followed by intramolecular cyclization. Time-course changes in BPS activity and xanthone formation were studied. Maximum product formation and enzyme activity were found at 12 and 9 h, respectively, after addition of the elicitor. The BPS cDNA was cloned using primers derived from *H. androsaemum* cDNA [2] and the open-reading frame was functionally expressed in E. coli as 6xHis-tagged protein. The enzymatic product after incubation with benzoyl-CoA and malonyl-CoA was identified as 2,4,6-trihydroxybenzophenone. Characterization of the recombinant enzyme is underway.

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B099

ARABINOGALACTAN-PROTEINS FROM CELL SUSPENSION CULTURES OF *PELARGONIUM SIDOIDES* DC

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Arabinogalactan-proteins (AGPs), secreted by suspension cultured cells of *Pelargonium sidoides* grown in different plant growth regulator-containing media were investigated quantitatively and qualitatively. Suspension cultures of *Pelargonium sidoides* have been established in MS media supplemented with 2,4-dichlorphenoxyacetc acid (2,4-D) or 2,4-D plus kinetin. The cell-free medium was used to isolate the AGPs by precipitation with β-glucosyl Yariv reagent. The pure AGPs have been structurally characterized with regard to the polysaccharide part. Quantification of neutral sugars by acetylation pointed out arabinose (Ara) and galactose (Gal) as dominating monosaccharide residues in a ratio of 1 : 2. Colourimetric determination of uronic acids revealed an amount of 5-8%. Linkage type analysis showed that the main components are 1,3,6-Gal(p), 1,3-Gal(p) and 1-Ara(f) as well as minor amounts of 1,6-Gal(p), 1,4-Gal(p),1-Gal(p), 1,5-Ara(f) and 1,2-Ara(f). Molecular weight of AGPs has been determined by size exclusion chromatography with laser light scattering detection and found to range between 80 and 85 kDa.

B100

XANTHINE OXIDASE INHIBITION BY DIFFERENT SAPONIN GLYCOSIDES FROM HYACINTHACEAE SPECIES

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The use of various Hyacinthaceae species especially these of genus Eucomis for medical applications has a longstanding tradition in the South African native population. Prepared decoctions from shaved bulbs and leafs in boiled water have been used for several indications in order to treat inflammation and pain.
Previous studies showed enrichment of pharmacological active metabolites in outlasting parts however in leaf extracts a highly decreased content was found [1].
Several human metabolic diseases e.g. gout and hyperuricemia are associated with elevated uric acid (UA), catalyzed by xanthine oxidase (XO) [2]. Hence therapy can be achieved by XO inhibition in order to block UA formation from hypoxanthine and xanthine, we determined inhibition of XO by Hyacinhaceae's saponin-glycosides. Allopurinol, a known substrate analogon of hypoxanthine and potent inhibitor of XO, has been frequently used for treatment of gout and hyperuricemia. Due to life-threatening side-effects of Allopurinol e.g. progressive renal failure or hepatitis, screening for novel non-purine derived XO inhibitors is an essential goal future pharmaceutical approach [3].
Extractions of bulbous parts from *Chionodoxa luciliae* Boiss., *Drimiopsis maculata* Lindl., *Eucomis bicolor* Bak., *E. pole-evansii* N.E.Br., *Ornithogalum dubium* Houtt. and *O. saundersiae* Bak. were separated and saponine-rich fractions were tested for inhibitory effects on XO activity.

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B101

EFFECTS OF SPIROCYCLIC NORTRITERPENOIDS FROM EUCOMIS COMOSA ON PEROXIDATION IN INFLAMMATORY PROCESSES

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The bulbous Hyacinthaceae *Eucomis comosa* (Houtt.) Wehrh. is known to be rich in homoisoflavones, chromanones and nortriterpenoids. In previous studies anti-inflammatory effects, especially the inhibition of cyclooxidases, have been described for substances and extracts of several Eucomis species [1]. The aim of our work was to investigate effects of isolated spirocyclic nortriterpenoids from bulbs of *E. comosa* on different inflammation parameters.
Inflammatory cells produce differentiation cytokines, which possess the ability to generate reactive oxygen species (ROS). ROS can damage cellular molecules which in turn augment the state of inflammation [2]. We tested inhibitory activity of *E. comosa's* nortriterpenoids on lipid peroxidation in erythrocytes from rabbit whole blood using the TBA *in vitro* assay. Furthermore inhibitory effects on the myeloperoxidase (MPO) from rabbit leukocytes were investigated. MPO serves as an essential enzyme for anti-bacterial responses in contaminated leukocytes [3]. As MPO levels are often increased in inflammatory processes, MPO contributes to the pathogenesis of chronic inflammatory diseases. Hence MPO inhibition would be beneficial in future anti-inflammatory therapies.

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B102

SULFATED POLYSACCHARIDES OF THE RED ALGAE *DELESSERIA SANGUINEA: "PROCESS DEFINES THE PRODUCT"*

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To increase the local fishery resources, a large-scale artificial reef was established in the Baltic Sea close to Nienhagen in 2003. Since the reef structures turned out to be abundantly colonized by the red macroalga *Delesseria sanguinea* (Hudson) Lamouroux (D.s.), a project was initiated to evaluate its economic applicability. D.s. was shown to contain substantial amounts of sulfated polysaccharides (D.s.-SP) exhibiting anti-inflammatory activity and anti-skin aging effects. Meanwhile more than 150 D.s.-SP batches were isolated from D.s. harvested over the period of 5 years, analyzed and tested. The D.s.-SP consist of a homogenous fraction of branched sulfated xylogalactans (gal : xyl = ~6.6), which can be isolated in reproducible, high quality by a standardized procedure. Depending on the harvest time of D.s., the D.s.-SP however may contain up to 23% glucose mainly representing starch. Although the latter does not influence the activities, it is regarded as an impurity. Therefore, the standard procedure was further modified to reduce the co-extracted starch as well as to generally increase the yield of D.s.-SP. According to the standard procedure, D.s. is extracted with demineralised water for 8h at 80°C yielding season-dependently 11.9% D.s.-SP (6.1-17.9%). As revealed by repeated 8h-extraction (EX), the D.s.-SP EX is not complete. Therefore, D.s. was extracted 2x for 4h and 4x for 2h, respectively. The 1st EX yielded 9.9% in both cases, by the 2nd EX the yields increased to 13.3% and 15.0%, resp. being higher than that by a single 8h-EX. The 4x2h-EX finally led to 17.9%, so that a 2x EX seems sufficient. The 2x2h EX is not only the shortest, but also results in the lowest glucose content (2x2h: 8.9%, 2x4h: 10.9%, 1st 8h: 14.4%, 2nd 8h: 25.1%). The use of 70% (v/v) ethanol or $CaCl_2$ solution instead of 90% (v/v) ethanol to precipitate the extracted D.s.-SP showed to be further measures to reduce the glucose content without significantly reducing the yield. The yield can be much increased by extracting dried and milled D.s. instead of fresh alga (e.g. 25.5% vs. 12.0%). In conclusion, the presented data on the isolation of D.s.-SP exemplary demonstrate the thesis "the process defines the product" claimed for plant extracts. Small changes of the procedure showed to significantly improve the quality of the D.s.-SP and to additionally increase their yield.

B103

A NEW HYALURONIDASE ASSAY: INLFUENCE OF VARIOUS ASSAY PARAMETERS ON THE INHIBITORY ACTIVITY

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Hydrolysis of connective tissue by enzymes such as hyaluronidase is a characteristic step during inflammation and tumor metastasis as well as during skin ageing. Accordingly, hyaluronidase represents an interesting target for the development of anti-inflammatory-, anti-metastatic-, and anti-ageing agents. The aim of the present study was to develop a convenient assay to screed potential hyaluronidase inhibitors.
The established "all in one microplate" assay consists of the following steps: (1) Incubation of hyaluronan, hyaluronidase and the test compound (hyaluronan degradation). (2) Cleavage of the terminal N-acetyl-glucosamine (GlcNAc) units from the resulting hyaluronan oligosaccharides by potassium tetraborate buffer, pH 10.0 (3) Incubation of the GlcNAc units with 4-dimethylaminobenzaldehyde (Morgan Elson reaction) (4) Measurement of the optical density at 570 nm. In the context of the assay development, the impact of following parameters was evaluated: (1) origin and salt form of the hyaluronan substrate, (2) composition of the buffer, (3) pH value of the buffer, (4) ion-concentration of the buffer. As exemplary hyaluronidase inhibitors unfractionated heparin (UFH), the semisynthetic glucan sulfate PS3, and the sulfated polysaccharide fraction from the red alga *Delesseria sanguinea* (D.s. SP) were used.
Ad (1): The extent of degradation turned out to depend on the origin of hyaluronan and increased in the order: human umbilical cord < bacterial fermentation < cock´s combs, whereas the respective inhibitory potency of test substances inversely improves, i.e. cock´s combs < bacterial fermentation < human umbilical cord. At pH 5.0, sodium hyaluronan showed to represent a slightly better substrate than potassium hyaluronan. This was confirmed (ad (2)) by performing the assay with potassium phosphate instead of sodium phosphate buffer. Ad (3): The inhibitory activities were found to be clearly dependent on the pH and increased in the order 7.0 < 6.5 < 5.5 < 5.0. Ad (4): As expected, high ion-concentrations of the buffer were associated with reduced inhibitory activities of the used test compounds.
In conclusion, the "all in one microplate" hyaluronidase assay is a useful tool for screening of potential inhibitors. To ensure its reproducibility, each parameter, especially the type of the hyaluronan, has to be standardized.

B104

COMPARISON OF THREE METHODS FOR THE DETERMINATION OF OSCS IN FALSIFIED HEPARIN

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A novel challenge in the quality control is the detection of counterfeit heparins. For that, we developed both a fluorescence assay (FA) and an anti-FXa assay-based method (aXa-A). After provisional revision of the Ph.Eur. monograph on heparin, the currently mandatory test for pharmaceutical industry is ^{1}H-nuclear magnetic resonance spectroscopy (NMR). The aim of this study was to compare our two methods with NMR.
A number of 150 samples of both pure and contaminated heparin batches (SH) were provided by the BfArM. Their OSCS content was determined using 3 methods. 1. FA: After enzymatic degradation of the heparin in the SH, the fluorescence intensity increase of Polymer-H by OSCS is measured as described earlier (Lühn, Alban, JPBA 2010). 2. aXa-A: The aXa-A determines OSCS by its heparinase-I (hep-I) inhibitory potency. After incubation of the SH with hep-I, the remaining heparin is measured in a chromogenic aXa-A. At both FA and aXa-A, OSCS is quantified by calibration curves with OSCS-spiked heparin. 3. NMR: NMR-spectra of the SH were recorded according to Beyer et al. (JPBA 2008).
All three methods matched concerning the categorisation of the 150 SH in non-, low-, middle- or high-contaminated samples. The correlation function of FA vs. NMR was $y = 0.9952x + 0.6567$ with a coefficient of determination $R = 0.9954$. In the aXa-A, saturation was observed in SH containing >10% OSCS. For SH with ≤10% OSCS, the correlation function was $y = 1.0844x + 0.1072$ with $R = 0.9934$. Further, some practical advantages of both FA and aXa-A compared to NMR became obvious: the required SH amount (< 30µg) is 1000 times lower, 30 SH are examined in the same time as 1 with NMR and has a much easier valid quantification procedure.
The study demonstrates that both the FA and aXa-A are as sensitive and reliable as NMR, but much less expensive. Thus, these two novel assays represent rapid and simple options for the detection of counterfeit heparins.

B105

SENSITIVE DETECTION OF HEPARIN MIMETICS BY MODIFICATION OF THE SENSOR MOLECULE-BASED POLYMER-H-ASSAY

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In 2008, unfractionated heparin (UFH) contaminated with the heparin mimetic oversulfated chondroitin sulfate (OSCS, up to 35%) penetrated the worldwide market and was associated with severe adverse reactions. Also batches of the LMWH enoxaparin contained up to 7% OSCS. Therefore, feasible and reliable methods to test heparins for falsification are urgently needed. We intended to elaborate a simple and rapid approach, which is based on a recently developed assay (Lühn, Alban, JPBA 2010) for direct quantification of heparins.
1. Basic microplate assay: Serial dilutions of UFH, enoxaparin, contaminated heparin, OSCS as an exemplary heparin mimetic and OSCS-spiked heparins, were mixed with a solution of the heparin sensor Polymer-H. After 10 min at room temperature, the fluorescence intensity (FI) was measured ($\lambda_{(em)}$ 330 nm, $\lambda_{(ex)}$ 510 nm). 2. Pre-treatment step: Incubation of the samples with heparinase I (hep-I). For complete degradation, several parameters (e.g. temperature, time, UFH-, hep-I conc.) were tested.
Like heparins, OSCS concentration dependently increased the FI of Polymer-H. Thus, it can be quantified, but not in the presence of heparins. Therefore, the heparin in OSCS-heparin mixtures has to be eliminated first. Enzymatic degradation with hep-I turned out to be most suitable. After optimization, the LOD was 0.4% / 0.5% OSCS in UFH / enoxaparin, and the LOQ 1.1% / 0.9%. The detected OSCS concentration was 0.01 µg/ml and so 50 times lower than expected. The reason is that OSCS concentration dependently inhibits hep-I resulting in incomplete heparin degradation. Thus the FI increase caused by OSCS itself is amplified by the remaining heparin.
This novel 2-step microplate fluorescence assay represents a sensitive, rapid and simple method to detect OSCS and other heparin mimetics in heparins. In contrast to ^{1}H-NMR spectroscopy, it requires neither expensive equipment nor much experience. Therefore, it could also be used in clinical practice, to check the applied heparin preparation when a patient suffered any adverse event.

B106

CHARACTERIZATION OF PHENOLIC COMPOUNDS BY HPLC-DAD/-ESI-MS^2 IN FLAVONOID ENRICHED EXTRACTS OF CURLY KALE

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Curly kale contains high amounts of ascorbic acid and other constitutional components like polyphenols. Especially flavonoids are known for antioxidative capacitiy and other beneficial effects to human health. Since type 2 diabetes belongs to the main diseases of the industrialized countries, the main concern of this project is to find flavonoids as potential inhibitors of the sodium glucose co-transporter (SGLT1) to reduce the intestinal uptake of glucose thereby avoiding increased postprandial blood glucose concentrations.
The flavonoid composition of curly kale (*Brassica oleracea* L. var. *sabellica* L.) and its extracts has been analyzed by HPLC-DAD/ESI-MS^2. Thereby, a large number of kaempferol and quercetin glycosides and their acylated derivatives could be found, furthermore structures of the flavonoid glycosides could be approved after specific cleavage of the ester linkage. Depending on the starting plant material three flavonoid aglycones could be quantified after acidic hydrolysis: kaempferol as the main aglycone, followed by quercetin and isorhamnetin.
The total flavonoid concentration in various sources of kale ranged from 1550 – 5000 ppm of fresh weight. In order to produce a final product with high flavonoid content an aqueous kale juice was prepared and concentrated using an adsorber resin (AMBERLITE™ FPX66). The identification of apigenin, rhamnetin and dihydrokaempferol (in traces) in such flavonoid enriched kale extracts with LC-ESI (+) MS has not been reported previously. Different enriched extracts of curly kale were tested for their inhibition of SGLT1 in *Xenopus* oocytes with two electrode voltage clamp technique. The inward current evoked by 1mM of the hSGLT1 substrate α –methyl-D-glucopyranoside was potently reduced.

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Poster
Pharmazeutische Chemie

C107

AUTODISPLAY OF NADH-OXIDASE YIELDS A CONVENIENT SYSTEM FOR COFACTOR REGENERATION

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Surface display of active proteins on living cells provides several advantages for biotechnological applications. Among other display systems in bacteria and yeast, autodisplay was developed based on the secretion mechanism of the autotransporter family of proteins and represents a very elegant way to express a recombinant protein on the surface of *Escherichia coli*. Using such cells as whole cell biocatalysts, a substrate to be processed does not need to cross a membrane barrier but has free access. Moreover, being connected to a carrier (the cell as a biological matrix), the surface displayed protein can be purified, stabilized and applied to industrial processes much more convenient than a free molecule.

Since the application of dehydrogenases in stereochemical synthesis is of growing interest, the simple supply of cofactors like NAD^+ becomes more and more important. One opportunity to regenerate NAD^+ from NADH is employing a NADH-Oxidase. These group of enzymes catalyses the oxidation of NADH with concomitant reduction of oxygen. The primary aim of the present study was to combine the advantages of autodisplay with the attractive features of a NADH-Oxidase from *Lactobacillus brevis*.
This enzyme in particular recycles NAD^+- cofactor by transferring 4 electrons and simply forming H_2O as a convenient by-product. NADH-Oxidase (NOX) was surface displayed on *E. coli.* Surface display was examined by its accessibility to proteases added to whole cells. NADH-concentrations in the presence of cells could be measured by the absorption at 340 nm. Testing parameters and enzymatic activity were optimized regarding an industrial application of the system.
The capability of the whole cell biocatalyst for cofactor regeneration in terms of stability was investigated. Cells stored at -20°C and -70°C turned out to be stable with no loss in activity for about seven weeks. To test its capacity for cofactor regeneration, the NOX-biocatalyst was combined with an aldehyde dehydrogenase using acetaldehyde as the substrate. In this combined assay, NAD^+ could successfully be regenerated by the whole cell biocatalyst. Our results show an economic and convenient way to regenerate cofactors by simply employing the whole cells.

C108

AUTODISPLAY OF COMBINATORIAL ANTIBODY LIBRARIES IN *E.COLI*

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Antibodies offer a high significance in therapy, as antitoxins or for diagnostical purposes. Thereby developing new antibody structures establish new applications and therapy strategies. One approach for developing new antibodies is to screen antibody libraries against a selected target structure. Such libraries consisting of variable antibody fragments are constructed by the autodisplay system. The autodisplay system is an established tool for expression of recombinant proteins at the cell surface of gram-negative bacteria. It is based on the autotransporter secretion mechanism, a mechanism naturally evolved for surface translocation of toxins or pathogenic factors. [1] Using this system, combinatorial antibody libraries of variable antibody fragments were expressed and displayed at the surface of *E.coli.* These antibody libraries were constructed by site-directed mutagenesis of the high variable complementary determining region 3 (CDR 3) of a mouse monoclonal antibody fragment. In order to gain higher variability, the CDR 3 sequence of the light chain and the heavy chain were mutated separately. Afterwards they were combined in one strain in order to co-express both variable regions without being connected via an internal peptidic spacer. The free motility within the outer membrane, a unique feature of autodisplay, enabled the formation of functional heterodimers. [2] The antibody library was screened against a selected target structure by fluorescence activated cell sorting. Binding to this target structure, a new antibody structure could be detected and was reanalysed by flow cytometer analysis.

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C109

AUTODISPLAY OF CYP106A2 AND CYP3A4 IN *ESCHERICHIA COLI*

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Cytochrome P450 enzymes are diverse oxygenation catalysts which can be found throughout nature. Even though most of the pharmaceutical industries interest has focused on drug development and biotransformation studies, these enzymes can also play an important role in the development of other useful chemicals [1]. At the moment there are two different ways to use these enzymes for synthetic purposes. They are either purified after recombinant expression and reconstituted with an artificial membrane system, or they are expressed and used in a whole cell context. Both ways have their limitations. Reconstituted membrane vesicles with P450 enzymes are laborious to produce and they are absolutely not suited for industrial applications. Using whole cells with intrinsic P450s limits the set of substrates to be converted to those which are able to cross membranes [2]. To circumvent these obstacles, CYP106A2 and CYP3A4 were expressed on the surface of *E. coli* cells by the use of Autodisplay, an efficient surface display system, developed in our group [3]. Cellular surface display allows the use of whole *E.coli* cells with the benefit that no purification or preparation steps of the target proteins are needed. The aim of the present project is to investigate whether it is possible to express a functional P450 enzyme on the surface of *E.coli* and in a later step conduct whole cell substrate converions .
For this purpose the genes of both enzymes were amplified by PCR and inserted into a plasmid encoding the domains needed for Autodisplay. Cellular surface display was prooved by fluorescence microscopy and fluorescence activated cell sorting (FACS). To investigate the functionality of the enzymes conversion assays were performed followed by a high performance liquid chromatography analysis. Our results indicate, that Autodisplay enables a functional surface display of P450 enzymes in *E. coli.*

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C110

AUTODISPLAY OF 60 KDA/ROSS-A AND DEVELOPMENT OF A SURFACE DISPLAY ELISA FOR SLE PATIENT SERA SCREENING

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To test human sera on an antibody reaction against a specific antigen enzyme linked immunosorbent assay (ELISA) is a common tool. Human 60 kDa Ro/SS-A is a well characterized nuclear antigen for autoantibodies which can be found in connective tissue diseases such as systemic lupus erythematosus (SLE). As in the case of human 60 kDa Ro/SS-A, antigens used in ELISAs are recombinantly expressed in *E. coli*. This means that cells have to be lysed and purification steps are needed in order to get the desired protein to set up the corresponding ELISA. To avoid these disadvantages human 60 kDa Ro/SS-A was expressed on the surface of *E. coli* using Autodisplay. Autodisplay is an efficient surface display system for gram-negative bacteria and is based on the autotransporter secretion pathway [1]. The cell surface exposure of 60 kDa Ro/SS-A was verified by immunolabeling of whole cells with a polyclonal serum against 60 kDa Ro/SS-A. Cells displaying the 60 kDa Ro/SS-A antigen on the surface were used to coat a 96 well microplate. 60 sera (30 patients and 30 control sera) were screened on a 60 kDa Ro/SS-A antibody reaction. In order to eliminate antibodies against native *E. coli*, human sera were preabsorbed with *E. coli* cells which were displaying a control peptide instead of 60 kDa Ro/SS-A prior to the assay. It turned out that 25 of the 30 control sera were negative, while 26 of the 30 patient sera showed a positive reaction. The new ELISA using *E. coli* with autodisplayed antigen showed a sensitivity of 86.67% and a specificity of 83.33% by a cut-off value of 0.28. These values are in the same range as those obtained with a commercially available ELISA using purified 60 kDa Ro/SS-A antigen. Our results show that Autodisplay provides a simple, rapid and cheap access to human antigens for an accelerated development of ELISAs in order to screen human sera against specific antibody reactions [2].

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C111

THE NEWLY DISCOVERED MOLYBDENUM ENZYME MARC IS INVOLVED IN THE REDUCTION OF *N*-HYDROXYLATED DNA BASES

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The mitochondrial Amidoxime Reducing Component mARC is a newly discovered molybdenum containing protein in eukaryotes. The human and plant genome codes for two mARC proteins. These 35 kDa proteins form the catalytic part of an enzyme system consisting of NADH cytochrom b_5 reductase, cytochrom b_5 and mARC.
The mARC-containing enzyme system is able to reduce the model substrate benzamidoxime and several *N*-hydroxylated prodrugs, like the *N*-hydroxypentamidine, *N*-hydroxymelagatran or guanoxabenz. Furthermore mARC is also involved in the detoxification of aromatic hydroxylamines formed as metabolites from the antimicrobial agents sulfamethoxazole and dapsone.
By cellular metabolism or the action of chemical and physical factors *N*-hydroxylated base analogues can be produced. In this report, the reduction of the toxic and mutagenic *N*-hydroxylated base analog *N*-hydroxy-cytosine to the corresponding amine cytosine is demonstrated. The activity of the reduction is compared with the model substrate benzamidoxime. We found also a high extrahepatic reductase activity in pig mitochondria from different organs like kidney or thyroid. The detoxification of base analogues could be a first hint on the physiological role of the mammalian mARC proteins.

C112

A NEWLY DISCOVERED HUMAN MOLYBDENUM ENZYME MARC - INVOLVED IN DRUG METABOLISM -

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mARC1 and mARC2 (mitochondrial amidoxime reducing component 1 and 2) are newly discovered mammalian molybdenum containing proteins, characterized by a so-called MOSC domain. These 35-kDa proteins represent a novel group of molybdenum proteins in eukaryotes as they form the catalytic part of a three-component enzyme system together with the electron transport proteins cytochrome b5 and its reductase. In mammals this *N*-reductive enzyme system is located in the outer mitochondrial membrane and it was already shown that it is responsible for the reductive activation of several *N*-hydroxylated prodrugs. Thus mARC plays a major role in drug metabolism though its physiological relevance is not still clear.
Unusual for drug metabolizing enzymes, we found unexpected high extrahepatic mARC-expression and activity levels, for example in kidney and thyroid. The determined reductase activities exceed even the hepatic activities, depending on the tissue investigated.
In continuation of our drug metabolism studies we show, that probably only the mitochondrial and not the microsomal isoform of cytochrom b5 is involved in described three component enzyme system. Furthermore the optimal stoichiometry of the recombinant enzymes cytochrom b5, its reductase and mARC was determined. By this an optimal reconstitution assay was developed which is now in routine use. Using this *in vitro* assay, we can predict if a new prodrug would be reduced *in vivo.*

C113

BENZAMIDOXIME METABOLISM IN FIVE GENETIC VARIANTS OF MITOCHONDRIAL AMIDOXIME REDUCING COMPONENT

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The oral bioavailability of amidines is limited. Therefore, amidoximes were introduced as prodrugs to increase bioavailability. They are less basic and not protonated under physiological conditions. This results in a sufficient oral absorption.

While studying *N*-reduction of these amidoxime structures, mARC (mitochondrial amidoxime reducing component) was recently found in our laboratory. It is the fourth human molybdenum containing enzyme and part of a mitochondrial enzyme system consisting of mARC, cytochrome b_5 and NADH cytochrome b_5 reductase. This enzyme system is capable of reducing *N*-hydroxylated compounds.

The human genome encodes two homologes of mARC, mARC-1 and mARC-2. Single nucleotide polymorphisms (SNPs) are known for both variants, but there is no data about their functional relevance.

We investigated four nonsynonymous SNPs in mARC-1 (c.493A>G, c.560T>A, c.736T>A and c.739G>C) and one in mARC-2 (c.730G>A) resulting in alterations of the encoded amino acid sequence. To determine the frequency of these SNPs they have been genotyped by pyrosequencing in a cohort of 334 healthy Caucasian individuals. Recombinant enzymes and variants have been expressed in *E. coli,* and *N*-reduction of benzamidoxime, a model compound for amidoxime prodrugs, has been used as activity assay for analyzing possible changes in substrate kinetics.

C114

A PHENOTYPIC YEAST ASSAY FOR THE SCREENING OF POTENTIAL AQUAPORIN INHIBITORS

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Aquaporins are membrane proteins that facilitate the diffusion of small uncharged solutes across biological membranes. Their selectivity ranges from strict water-selectivity to permeation by larger molecules such as glycerol. They are found in nearly all organisms. In humans, they enable rapid fluid homeostasis throughout the body, a prominent example of dysregulation being Diabetes insipidus, in which the trafficking of aquaporin 2 is affected.
The determination of the three dimensional structures of some aquaporins has allowed the in silico high-throughput screening of molecules to find potential inhibitors.
We use a phenotypic yeast assay to test the activity of potential aquaporin inhibitors. A yeast strain unable to grow on ammonium salts as the sole nitrogen source will do so if it is made to express an ammonia-permeable aquaporin. An inhibitor is expected to reduce yeast growth. An aquaporin-independent nitrogen source, such as an amino acid, serves as a control. Liquid yeast cultures are incubated inside microtiter plates and their growth recorded turbidimetrically.
So far we have tested a small number of potential inhibitors of Plasmodium falciparum aquaporin, with no “hit” as yet. Further candidates as well as potential inhibitors of human aquaporin 9 will be tested.

“A yeast-based phenotypic screen for aquaporin inhibitors”
B. Wu, K. Altmann, I. Barzel, S. Krehan, E. Beitz; Eur J Physiol 456, 717-720 (2007).

C115

DEVELOPMENT OF A CE BASED ASSAY FOR DETERMINATION OF HUMAN MYT1 KINASE ACTIVITY

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The human Myt1 kinase (Myt1hu) is an enzyme that catalyzes the phosphorylation of threonine 14 and tyrosine 15 of Cdc2 kinase by transferring the γ-phosphate group from ATP to the hydroxyl group of the threonine, or tyrosine residue of the target protein. The inhibitory phosphorylation of Cdc2 is important for timing the entry into mitosis (M phase). The transition from G_2 to M phase requires the activity of M-phase-promoting factor (MPF), which consists of Cdc2 and B-type cyclins (cyclin B1). Inhibition of Myt1 kinase is known to cause premature activation of Cdc2. Since inhibitors of Myt1 kinase are supposed to kill rapidly proliferating cells and interfere with cell cycle checkpoints, such inhibitors are potential new targets for drug development and could represent a valuable addition to conventional chemotherapy in order to help overcoming resistances. Therefore, determination of the kinase activity requires development of new methods in biochemical research as well as in drug development. Here we describe a CE based kinase assay for human Myt1 using a fluorescence detection method for determination of phosphorylation status of the amino acids tyrosine and threonine in a specific peptide fragment of Cdc2. Under the CE conditions used, the different phosphorylated forms of the peptide were rapidly separated within 15 min. The quantification of the phospho-peptides enables us to characterize the human Myt1 kinase activity and also allows conclusions about kinetic parameters. We prepared a fluorophore-labeled substrate QKIGEGTYGVVYKC peptide which is a fragment of Cdc2 as well as the mono- and bis-phosphorylated forms as references. The results were quantified by the areas of the fluorescence peaks and highlight the feasibility of this CE method, which is a simple and reliable technique for determining and characterizing various enzyme reactions particularly kinase enzymes.

C116

IN VITRO INVESTIGATIONS OF NEW BRANCHED LIPIDS FOR LIPOSOMAL GEN TRANSFER

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Three major aspects can characterize an ideal way of delivering DNA into cells. At first the transgene should be protected against the degradation through cell nucleases. Additionally the gene material has to be transported high efficiently through biological membranes and other cytosolic compartments with no or less noxious effects. These aspects have been investigated intensively over the past few years demonstrating that the finishing line to find the perfect gene transfer method could not be already crossed. Two different methods have been taking shape. Vector-mediated transfer by liposomes, polysomes and virus particles and the non vector-mediated gene delivery by microinjection, biolistic transfection or electroporation, which can be summarized as physical and mechanical methods, are only few examples for these two different groups. Despite of simplicity of use and lack of toxicity non vector-mediated transfer systems suffering from less satisfying results for gene expression based on fast DNA degeneration and the restriction of application for certain tissues new usefull alternative vector-mediated transfer systems had to be developed.
There are two different vector-mediated delivery systems. Viral vectors are characterized by high efficiency for gene delivery but also by the possibility of causing strong immune responses and the limitation of transgene-size. Non-viral gene transfer with cationic liposomes provides the delivery of genetic material with low toxicity and high DNA-loading capacity and reproduceability.
We use new synthesized cationic lipids with different head and backbone groups combined with different helper lipids like DOPE or cholesterol to form lipoplexes able to surpass LipofectAmine™, used as reference.Transfection efficieny and cytotoxicity were analyzed in serum-free and serum-containing medium with MTT, detecting the cell viability, and the ONPG.-assay measuring the galactosidase activity after incorpoation of the Lac-Z-gene containing plasmid DNA.

C117

SYNTHESIS OF NOVEL CATIONIC LIPIDS WITH MALONIC DIAMIDE BACKBONE AND LYSINE CONTAINING HEADGROUP

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Gene therapy based on the introduction of genetic material into cells in order to obtain a therapeutic benefit is a promising method for the treatment of genetic disorders but also for cancer, cardiovascular, and neurologic diseases. To realise the gene transfer special delivery systems (vectors) are used. At present viral gene delivery systems dominate in clinical trials. But due to the drawbacks using viral vectors which are not negligible the development of non viral gene delivery systems is a promising alternative. Non viral vectors are less immunogenic than the viral ones and they do not induce cancer. However, the toxicity and the low transfection efficiency of these systems are still problematical and require new developments and new substances in this field.
One promising field of non viral gene transfer is the lipofection. Following the potent lipids with malonic diamide structure, designed in our research group, we synthesized new compounds with an enlarged cationic headgroup structure. It was realised by the coupling of the basic amino acid lysine via tris(aminoethyl)amine spacer to the malonic acid amide backbone. Furthermore, we varied the length and degree of saturation of the alkyl chains attached to the malonic acid structure establishing structure-function relationships. The new compounds were tested in cell culture systems (LLCPK1 and A549) to investigate the in-vitro transfection efficacy and toxicity properties compared to a commercially available transfection reagent.

C118

PREDICTED INTESTINAL PERMEABILITIES VS. IN-VIVO AVAILABILITIES OF PEG 400 OLIGOMERS

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Polyethylene glycols are polymers prepared by polymerization of ethyleneoxide. They are of importance as cosolvents in drug preparations. PEG 400 represents a mixture of different oligomers with an average molecular weight of 400 and most abundant oligomers between mol. wt. 238 and 643. PEG 370 and PEG 414 oligomers are the major components (19.83 and 19.44 %, respectively). PEGs present in the organism, however, appear to exhibit a smaller influence on disposition processes than surfactants, such as Tween 80 and Cremophor EL, although, e.g., P-gp mediated transport and verapamil metabolism were affected to some extent.
Because of the wide range of molecular sizes it is anticipated that effective intestinal permeabilities (Peff) are different and so should be oral absorption. Effective permeabilities were estimated for man and rat along with logP values employing Gastroplus/ADMET Predictor. In-vivo absolute bioavailabilities are based on rat data, where PEG 400 (0.5 ml/kg = 575 mg/kg) was dosed intravenously and perorally, respectively, and where no interconversion was assumed.
In-silico Peff ‘s (cm/s x 10^4) for man are ranging from 0.98 (PEG 238) to 3.39 (PEG 634), for rat from 0.24 (PEG 238) to 1.04 (PEG 634) and correlate with the logP values (Moriguchi model) of -1.19 up to -3.49. Kinetic analysis of disposition parameters indicates higher initial volumes of distribution with higher molecular weight. Systemic availabilities for a p.o. dose increase along with molecular weight and lipophilicity from 40.9 % (PEG 238) to 72.4 (PEG 414), but decrease beyond 414 down to 18.8 % (PEG 643).
A decrease in the fraction absorbed and true Peff‘s appears to be the most reasonable explanation for the discontinuity of the correlation between in-silico Peff and in-vivo oral availability.

C119

ACTIVATION OF MATRIPTASE-2 IN HEK CELLS IS A TRANS-MECHANISM

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Matriptase-2 is a recently identified member of the Type II Transmembrane Serine Protease (TTSP) family, an emerging class of cell surface proteases involved in tissue homeostasis and several human disorders.[1]
Matriptase-2 is predominantly expressed in the liver and recently the physiological role of matriptase-2 as a key regulator in iron homeostasis was identified.[2,3] A correlation between mutations in the gene encoding matriptase-2 and iron-refractory iron deficiency anemia (IRIDA) was found. Furthermore, it was demonstrated that a lack of matriptase-2 was linked to the inability to suppress expression of hepcidin, the systematic regulator of iron homeostasis. Matriptase-2 downregulates high levels of hepcidin through proteolytic processing of hemojuvelin, a membrane-bound protein promoting hepcidin expression.
To gain further insight into the activation of matriptase-2, we used HEK293 cells stably transfected with cDNA encoding human matriptase-2 and encoding the inactive variant of matriptase-2 in which the catalytic triad Ser-753 was exchanged for alanine (S753A).[4] We could demonstrate that the cleavage of the zymogen to form the mature enzyme does not occur if the protease is mutated at the proteolytic site. This indicates that matriptase-2 activity is necessary for its own activation and that the enzyme undergoes an autocatalytic activation process.
To analyze whether the autoactivation process is catalyzed by the same (cis-mechanism) or by another (trans-mechanism) matriptase-2 molecule, we co-transfected HEK293 cells with two vectors, one expressing His-tagged wild type matriptase-2 and one the c-Myc-tagged inactive S753A matriptase-2 variant. Activation cleavage of the c-Myc-tagged inactive S753A mutant form was catalyzed by His-tagged wild type matriptase-2 suggesting a trans-mechanism in which matriptase-2 activates one another.
Thus, our results show that activation of matriptase-2 is an autocatalytical process via a transactivation mechanism.

[1] Velasco et al. 2002, J. Biol. Chem. 277, 37637-37646
[2] Finberg et al. 2008 Nat. Genet. 40, 569-571
[3] Du et al. 2008, Science 320, 1088-1091
[4] Stirnberg et al. 2010, Biochem. J., in press

C120

QUANTITATIVE DETERMINATION OF XANTHURENIC ACID IN ERYTHROCYTE LYSATES USING LC/ESI/MS/MS

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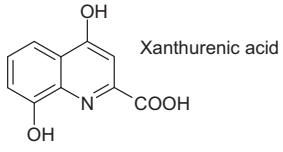
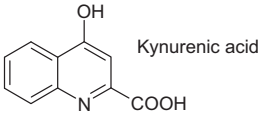
The unicellular malaria pathogen *Plasmodium falciparum* is transmitted to its human host by blood feeding female mosquitoes of the genus *Anopheles*. Hence, development of the parasites in the mosquito represents an indispensable prerequisite for completion of the parasite’s life cycle. It starts with the uptake of erythrocytes containing mature male and female sexual precursor cells, the gametocytes, by the blood-feeding mosquito. In the mosquito midgut gametocytes are activated both by a drop in temperature and by presence of the mosquito-derived molecule xanthurenic acid (XA). The activation of gametocytes leads to egress from the erythrocyte and the subsequent formation of micro- and macrogametes, which then undergo fertilization. It still remains unclear, which role XA plays during signal transduction leading to gametocyte activation. Here we report that XA interacts with red blood cells independent of infection and preliminary data suggest a transport of this molecule across the erythrocyte membrane. In order to investigate the factors involved in the erythrocytic uptake mechanism of XA, we measured XA concentrations in erythrocyte lysates after incubation of erythrocytes with different concentrations of XA with and without substrates of amino acid transporters. For this purpose, we developed a liquid chromatography tandem mass spectrometry method with electrospray ionization (LC/ESI/MS/MS) which allowed the precise and fast quantitative determination of XA and the internal standard kynurenic acid (KA) in erythrocyte lysates. Both XA and KA were measured in the positive ESI mode and linearity of the reversed phase LC/ESI/MS/MS method for XA was between 0.5 and 250 ng/ml.



Xanthurenic acid



Kynurenic acid

C121

REVIEW AT A GLANCE: DIFFERENT APPROACHES TOWARDS PRECISE PROTEIN QUANTIFICATION

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Precise protein quantification is still challenging. Several approaches have been introduced during the last years, e.g. mass spectrometry (MS), gel electrophoresis (GE), capillary electrophoresis (CE), microfluidics. In general, a technical error of up to 20% is assumed. However, especially for quality control and for proteomic researches high precision is essential. We compare the most common techniques for protein analyses in term of reproducibility. In addition, the advantages and limitations of each method are presented. In recent publications, low percent relative standard deviations (RSD%s) of <3% were reported for protein quantification by MS. Moreover, by one-dimensional GE RSD%s of 1.5 - 3% were achieved under favourable circumstances. More studies have to be done to make precise protein quantification feasible.

C122

A STUDY OF INFLUENCES ON PROTEIN PROPERTIES USING AFFINITY CAPILLARY ELECTROPHORESIS

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Understanding the unique properties of proteins is essential. However, the knowledge about their physico-chemical properties and their real affecting size is still insufficient. Recently, findings that small proteins do not fit into large pores have been reported [1]. Their hydrodynamic radius can be measured by various methods, e.g. by Dynamic Light Scattering (DLS) or Atomic Force Microscopy (AFM) where similar values for the radius are achieved. Because this hydrodynamic radius is much smaller than the above mentioned pores there must be an expanded affecting size of the proteins. Therefore the influence and behavior of the solvent layers and of various reagents on protein properties have to be thoroughly discussed. Affinity Capillary Electrophoresis (ACE) was used to measure the influences of various reagents (e.g. different salts) on proteins. By varying the reagent concentration in the buffer solution a mobility shift can be observed as a result of complexation between the analyte and the protein. These changes in electrophoretic mobility can also be used to determine the binding constants of the reagents to the proteins.

[1] Edward Yeung, presentation on HPLC 2009 in Dresden

C123

DRUG ANALYSIS IN THE PRESENCE OF MATRIX PROTEINS: VALIDATION OF A BLOOD-BRAIN BARRIER MODEL BY DIRECT-INJECTION MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Capillary electrophoresis (CE) as a well-suited analytical technique especially when proteins are involved has established in clinical laboratories for more than a decade. A micellar electrokinetic chromatography (MEKC) method with direct sample injection has been developed in order to characterize and validate a blood-brain barrier (BBB) model by testing drug permeability. An aqueous stock solution of each drug (acetaminophen, caffeine, carbamazepine, cimetidine, indometacin and propranolol) was diluted with culture medium (Quantum 286, PAA Laboratories, Pasching, Austria) to a final concentration of 0.1 mg/mL and transferred to the BBB model. After six hours, the samples were analyzed by CE without any further sample pre-treatment. All drugs are stable at the test conditions. Proteins from the culture medium did not impede the analysis, as the drug-protein interactions were reliably suppressed by high concentration of sodium dodecyl sulfate (200 mM) in the running buffer (borate buffer, 60 mM, pH 10.0). A standard fused-silica capillary (ID: 50 µM, l_{tot}: 50 cm, l_{eff}: 42 cm) has been cleaned by a mixture of equal parts of running buffer and isopropanol after each run. This rinsing reagent could very effectively remove adsorbed proteins from the capillary wall and avoid capillary blocking, thus the capillary could be continuously used for more than a month. As the result of a careful optimization, a voltage of 30 kV was found to be the most suitable as it allows short analysis time as well as a sharp separation. The limits of detection were approximately 10 ng/mL.

C124

HIGH PERFORMANCE SIZE EXCLUSION AND STRONG ANION EXCHANGE CHROMATOGRAPHY FOR PROTEIN ASSAYS

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The number of protein based pharmaceuticals, such as monoclonal antibodies (mab), increases steadily, since they were introduced as therapeutic substances. Undoubtedly, one main pillar of the quality control (QC) for these biologicals is an unfailing method for quantitative analysis of the API. Classic quantification methods for proteins (e.g. Bradford assay or gel electrophoresis) may be sufficient for bioanalytical assays but unfortunately they are inappropriate for QC purposes due to their low selectivity and low precision, respectively. Therefore intention of this work is to present two reliable and inexpensive alternatives. One of these is High Performance Size Exclusion Chromatography (HP-SEC / SE-HPLC). It will be shown that the application of the non-ideal SEC mode [1] not only allows for increased efficiency but also for precise quantification of the model proteins Ovalbumin and Myoglobin. The second method is Strong Anion Exchange (SAX). It allows for very fast separation and quantitation (approx. 5 min) of the model proteins Ovalbumin, Myoglobin and BSA. Both methods yield data of high precision (about 1% RSD each for retention times and peak areas) and can achieve low quantitation limits (< 15µg/ml for SAX and < 4µg/ml for SEC), depending on the used detector (two different UV detectors were used in these experiments). In the following, both methods will be compared in terms of precision, practicability, effort during sample and mobile phase preparation, selectivity, overall results and total analysis time (including post processing of raw data).

[1] Kopaciewicz, W. and Regnier, F.E. *Anal. Biochem.* 126 (1982) 8 – 16

C125

NANOLIQUID CHROMATOGRAPHY/MASS SPECTROMETRY OF NATIVE AND BIOTINYLATED GLYCANS ON A POROUS GRAPHITIZED CARBON-CHIP

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About 50% of all proteins are supposed to be glycosylated and they seem to be involved in all kinds of cell processes. Depending on the structure, glycoproteins do not only participate in physiological activities. Moreover, they are associated with pathological pathways as well. Therefore, there is a great interest in identifying glycans, particularly, in changes in the glycan structure, which are presumed to occur at diseases like cancer, immune deficiencies, etc.
Consequently the improvement of analytical methods and development of instrumentation in order to produce reliable and specific data about the protein glycosylation is very important. This is challenging due to both the high variety and complexity of sugar chains and small sample amounts.
In this work, glycans from different glycoproteins have been analyzed by means of a nano-LC/Chip-mass spectrometry.
Ovalbumin, ribonuclease from bovine pancreas (RNase B) and alpha-1-acid glycoprotein (AGP) were deglycosylated with PNGase F and extracted carbohydrates were derivatized with 2-AB (2-aminobenzamide) and BACH (biotinamidocaproyl hydrazide). Then, analysis was performed with a nano-LC/ESI-ion trap system equipped with a chip cube (Agilent). Separation was acquired on a porous graphitized carbon chip with methanol and water as solvents. With ovalbumin, RNase B and AGP, all three types of N-glycans (hybrid, high-mannose, complex) were analysed in native form and as 2-AB- and BACH-derivatives. In particular, the fragmentation behaviour upon MS/MS was observed. Through identifying the generated fragments of the sugar chains it is possible to draw conclusions about the original glycan structure. Especially, ring cleavages are of avail as they provide more specific structural information. Here, the number of these cleavages was increased by means of derivatization. Particularly, the BACH-derivatization showed a high occurrence of ring fragments in comparison to the native and AB-derivatized carbohydrates. Implicating the capability of BACH to link with avidin/streptavidin, it is clearly a great tool for the analysis of unknown glycans.

C126

VALIDATION OF A NEW HPLC-UV METHOD FOR THE SEPARATION OF METHIONINE AND ITS RELATED SUBSTANCES.

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L-Methionine is used for pharmaceutical applications and DL-methionine for supplementation in animal food. Currently many HPLC methods are known to quantify methionine. Most of the methods work with a derivatization of the primary amino function. No selective method is available to detect the methionine, the L-methionine sulfone and the L-methionine sulfoxide, the N-acetyl-methionine and the N-acetyl-methionyl-methionine dipeptides in one run. The TLC method of the European Pharmacopoeia applies ninhydrine as derivatization reagent and the maximum limit for each impurity is set at 0.5% in L-methionine and 0.2% in DL-methionine batches. In this investigation a new and robust HPLC-UV method to separate methionine from its related substances has been developed and validated. The separation of the afore-mentioned impurities was achieved by means of a Phenomenex C_{12} Max-RP column using a mobile phase composed of aqueous 25mM heptane sulfonic sodium salt solution and acetonitrile = 85:15. The pH of the water phase was adjusted by phosphoric acid 85% to 2.0. Flow rate of the mobile phase was set at 1.5 ml/min and the UV- spectrophotometer was set at 190 nm. The column was thermostated at 25 °C. Methionine and all impurities could be baseline separated. Good linearity for all analytes was achieved in the range of 0.005% to 1%. The sum of impurities for all the examined batches was 0.8%. Stability testing revealed that the solutions for the HPLC analysis have to be prepared freshly and protected from sunlight and heat.

C127

TESTING OF ULTRAFILTRATION CELLS MADE OF POLYVINYLIDENE FLUORIDE FOR DETERMINATION OF PROTEIN BINDING OF QUATERNARY AND BISQUATERNARY COMPOUNDS

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The extent of protein binding is one of the key parameters in both pharmacodynamics and pharmacokinetics. High protein binding extend the risk of drug interaction because of displacement of other drugs especially one with also a high protein binding and a low therapeutic index. Thus new developed drugs need to have a low protein binding. In our research group we use the continuous ultra filtration developed by Kinawi and Teller and latterly optimized by Heinze, Albert and Holzgrabe for his purpose. Since new developed quaternary and bisquaternary compounds as new antiinfectives couldn't be measured by this method, further improvements were needed. As the tested compounds eluted too slow through the ultra filtration cell it was suggested that there is a strong binding of the compounds to the cell material poly(methyl methacrylate). To reduce the unspecific binding, surface coating and other cell materials were tried. Surface coating was not a suitable method because of damages to the cells by the coating chemicals. So polyflurated plastics as cell material were tested. Polytetrafluoroethylene showed good chemical indifference but the material was to supple so the cells wouldn't last long. Finally polyvinylidene fluoride was found to be most suitable, concerning to its hardness and chemical indifference. Eventually the quaternary compound GB-AP-05, which wasn't measurable with poly(methyl methacrylate) cells, could be successfully tested by using the cells made of polyvinylidene fluoride.

C128

TRIAMTERENE IN LIVER DYSFUNCTION: FAST ASSAY FOR HYDROXY METABOLITE-TO-DRUG RATIO IN PATIENT URINE BY CAPILLARY LC

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As for caffeine and lidocaine a strong dependence of the ratio of hydroxyl metabolites to parent triamterene and the stage of liver disease was detected. With respect to its therapeutic use, triamterene is a potassium-sparing diuretic that is used for antihypertensive treatment. It acts through inhibition of the epithelial sodium channel (SCNN1) located at the luminal side of the collection tubule. Being a CYP1A2 substrate with no alternative clearance route triamterene is para-hydroxylated and this is restricted to the liver. Hxdroxylation as rate-limiting step in triamterene kinetics in man is followed by a rapid sequential step, which is formation of the respective sulfate conjugate.
In order to estimate liver function through triamterene clearance routinely as a contributional factor in liver differential diagnosis, availability of a quick, reliable, and cost-efficient procedure is essential.
A suitable CLC-stationary phase was a 20 cm capillary with an inner diameter of 200 µm and packed with RP-18 coated 5µ-particles. With a mobile phase consisting of acetonitrile/water pH3.2 containing dodecylamine (12/88, v/v) and delivered at a flow rate of 5 µl/min total analysis time was below 10 min. Capacity factors were 1.42 and 3.91 for triamterene and its major metabolite. Detection of the analytes in the eluate was via fluorescence measurement at 365/449 nm. With an injection volume of 80 nl (direct injection of urine supernatant) LOQ was appropriate for assaying triamterene and its major metabolite in patient urine. Elution order was reverse without dodecylamine addition. In this case the respective capacity factors were 4.90 and 2.54.

C129

AGING OF DRUG PRODUCT MATRIX AS A POTENTIAL CAUSE OF MASS IMBALANCE

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An important quality feature of stability testing of drug products is mass balance [1]. One possible cause of mass imbalance is the cascading degradation of the drug substance, eventually leading to the formation of degradation products with concentrations below the limit of detection. The question whether the aging of the formulation matrix influences the recovery of substances in low concentrations and therefore the mass balance, has so far not been considered. However, this is of particular relevance in case of the detection of genotoxic substances in trace amounts. Complete recovery of these substances – as well as of any other relevant degradation product – is desirable at any time of the storage period. The objective of the present work was the investigation whether the aging of matrix simulated by various stress tests influences the recovery of genotoxic substances in trace amounts. The genotoxic substance 4-chloroaniline (PCA) was used as a model substance. In a first step, an analytical method was developed, allowing the identification of PCA in commercially available tablets with proguanil (Paludrine®), and the determination of PCA with low limits of detection and quantification (LOD and LOQ). A HPLC method was developed using an Ascentis Express C18 analytical column. The mobile phase consisted of acetonitrile and phosphate buffer (pH 7). For chromatographic separation a gradient was used. An ultraviolet spectrum, a fluorescence spectrum and a mass spectrum were performed to identify PCA. For PCA quantification, the HPLC method was coupled with a fluorescence detector, using external calibration. LOD and LOQ of PCA were found to be 0.003 µg/mL and 0.01 µg/mL, respectively.
[1] R. Kirsch, Stabilität von Norethisteron® Tabletten unter Berücksichtigung der Massebilanz, Diss., Friedrich-Schiller-University, Jena, 2005

C130

INFRARED THERMOMETRY IN CAPILLARY ELECTROPHORESIS

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Capillary electrophoresis (CE) is a well established and frequently used analysis technique in the development and quality control of pharmaceuticals.
An unavoidable problem in CE is the generation of heat depending on the applied voltage and the resulting electric current flowing through the electrolyte. The arising temperature in a CE system has effects on the stability of migration times and peak areas.
Infrared thermometry is used to monitor the Joule heating. The thermometer collects the temperature data of surface areas. So the temperature is measured on the outside of the capillary. The received data is compared to temperature data calculated with different methods by [1] and [2]. The data of the infrared thermometer has to be corrected because the used methods calculate the temperature inside the capillary. The results suggest that the temperature of a CE system should be monitored during laboratory routine. In addition, the maximal suitable power per meter is estimated.

[1] Evenhuis, C. J.; Guijt, R. M.; Macka, M.; Marriott, P. J.; Haddad, P. R: Electrophoresis, 26, (2005), S. 4333–4344.
[2] Knox, J. H.; McCormack, K. A.: Chramatographia, 38, (1994), 3/4, S. 207–214.

C131

BIODISTRIBUTION STUDIES ON GOLD COORDINATION COMPOUNDS BY TXRF SPECTROSCOPY

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X-ray fluorescence (XRF) appears when atoms are irradiated with short wavelength X-rays. Due to the fact that emitted fluorescence photons are characteristic in wavelength and energy, a specific elemental analysis and quantification using XRF as a non-destructive analytical technique is possible even in complex multielement matrices. The classical XRF is not suitable for trace element analysis in a cellular environment because of strong matrix effects.
Technical modifications of the excitation mode established total reflection X-ray fluorescence (TXRF) spectroscopy as a new analytical technique initially applied to environmental research [1].
As a promising alternative method for biodistribution studies we compared TXRF spectroscopy with the well established atomic absorption spectroscopy (AAS) aiming to identify advantages and disadvantages of both methods. For this purpose gold coordination compounds were measured in aqueous and biological environments and their biodistribution was evaluated. The current results of this ongoing project will be presented.

[1] Elemental Analysis of Environmental Samples by Total Reflection X-Ray Fluorescence: a Review, R. Klockenkämper, A. von Bohlen, X-Ray Spectrometry 25, (1996) 156

C132

A FACILE NOVEL SYNTHESIS OF FINGOLIMOD ANALOGUES

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The role of sphingolipids in chemical biology as important modulators of diverse (patho)physiological functions has raised increased therapeutic interest. Within the sphingolipid pathways different important potential targets such as S1P receptor subtypes, sphingosine kinases, ceramide synthases are actually widely explored. Fingolimod (FTY720, Gilenia®) as parent prodrug compound for S1P receptor agonist is the most advanced drug in this group.

Numerous related synthetic approaches for FTY720 have been described in literature and in patents, but in our hands none of those offered preparative approaches was practical enough for the synthesis of FTY720 or its derivatisation. For the development of FTY720 derivatives as a pharmacological tool its oxy-analogue (R)-AAL can be taken as comparable lead structure. The goal of our approach was to provide easy access to the aromatic ether analogues to FTY720 (O-FTY).

We have developed a new efficient multigram synthesis of the oxy-FTY720 analogue in four steps without any chromatographic separation and with overall yield of over 50%.[1]

This study was kindly supported by LOEWE Schwerpunkte LiFF and OSF.

Literature:
[1] A. Zivkovic and H. Stark, *Tetrahedron Letters* **2010**, *51*, 3169-3171.

C133

STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON 1-INDOL-1-YL-PROPAN-2-ONES AS DUAL INHIBITORS OF cPLA$_2\alpha$ AND FAAH

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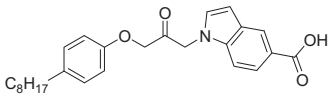
In mammalian organism, derivatives of arachidonic acid play important roles as algesic and pro-inflammatory as well as analgesic and anti-inflammatory mediators. On the one hand, oxidation products of arachidonic acid such as prostaglandin E_2 and leukotriene B_4 formed via the arachidonic acid cascade are involved in the pathophysiology of pain and inflammation. On the other hand, the arachidonic acid amide anandamide generated via the endocannabinoid pathway has analgetic and anti-inflammatory properties. The key enzyme in the formation of oxidized derivatives of arachidonic acid is cytosolic phospholipase $A_2\alpha$ (cPLA$_2\alpha$). An important enzyme in the endocannabinoid metabolism is fatty acid amide hydrolase (FAAH), which rapidly inactivates anandamide by cleavage to arachidonic acid and ethanolamine. Therefore, inhibitors of both cPLA$_2\alpha$ and FAAH may represent new agents against pain and inflammation.



1

Recently we have found that the indole-5-carboxylic acid derivative **1** is a dual inhibitor of cPLA$_2\alpha$ and FAAH. In course of structure-activity relationship studies, the indole part of the molecule has been varied.[1] In further studies we replaced the carboxylic group of **1** by bioisosteric residues such as amide, sulfonamide and different heterocycles. The results of these structural variations of **1** on inhibit-tory potency against both enzymes are presented.

Literature:
[1] Forster, L.; Ludwig, J.; Kaptur, M.; Bovens, S.; Schulze Elfringhoff, A.; Holtfrerich, A.; Lehr, M. *Bioorg . Med. Chem.* **2010**, *18*, 945-952

C134

A NOVEL PHARMACOLOGICAL TOOL: FLUORESCENT CELECOXIBDERIVATIVES

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Celecoxib, 4-(5-*p*-tolyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)benzenesulfonamide, a cyclooxygenase-2 (COX-2) selective inhibitor, belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs). By inhibiting the COX-2 isoform only – celecoxib shows an IC_{50} value of 0.87 µM – the gastrointestinal side-effects of traditional NSAIDs are faded out. Despite this benefit of COX-2 inhibitors they still reveal serious side-effects on the cardiovascular system[1,2].
Beside the anti-inflammatory, analgesic and antipyretic effects, celecoxib also shows COX-dependent and COX-independent anti-carcinogenic effects, which actually are far from being understood.[2] Surprisingly the concentrations necessary for these effects are 20-times lower in *in-vivo* than in *in-vitro* assays, which is potentially due to celecoxib accumulation in distinct cell compartments.[2] With use of fluorescence-labeled celecoxib derivatives as novel pharmacological tools the accumulation hypothesis and the interaction partners of celecoxib within cancer cells should be studied and tested.
Based on known structure-activity-relationships (SAR) and molecular docking-experiments on celecoxib and related compounds, a set of different fluorescence-labeled celecoxib derivatives was designed, synthesized and tested for activity. 1-(4-Sulfamoylphenyl)-5-*p*-tolyl-1*H*-pyrazole-3-carboxylic acid was successfully coupled with dansylated linker to yield fluorescence-labeled celecoxib analogues with substitutions at the position of the former trifluoromethyl group. In addition to that, the fluorophore was connected with the former tolyl group via carboxamide and ether functionalities, respectively.
Preliminary experiments with these novel pharmacological tools showed that they are able to pass the cell membrane and, upon excitation, can be visualized within the cell. Further data on cellular location are awaited.

Acknowledgements: Kindly supported by LOEWE Schwerpunkte LiFF and OSF.
Literature:
[1] S. Grösch et al., *J Natl Cancer Inst* **2006**, *98*, 736.
[2] I. Tegeder, G. Geisslinger, *Naunyn-Schmiedeberg's Arch Pharmacol* **2006**, *373*, 1.

C135

AZA-ANALOGUES OF DIBENZEPINONES AS P38 MAP KINASE INHIBITORS

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Inhibition of p38 MAP kinase is a valid approach for the treatment of inflammatory and autoimmune diseases.
Prototype inhibitors of p38 MAP kinase like SB 203580 are well investigated lead compounds but often suffering from high toxicity and poor selectivity, making novel chemotypes necessary.
Distinctly different templates are linear binders, e. g. benzophenones or ketopyrazoles. Starting from benzophenone-lead compounds (Ottosen et al., 2003) our hypothesis was to design more rigid structures by linking the two aromatic systems, which led us to substituted dibenzepinones.
Although these compounds showed high potency in p38 enzyme assays, they are highly lipophilic and poorly soluble in aqueous media and may therefore suffer from low bioavailability. Our strategy was to improve aqueous solubility by using more hydrophilic scaffolds. Better physicochemical properties could be achieved by synthesis of aza-analogues of above-mentioned dibenzepinones compromising affinity to the enzyme.

C136

5-LIPOXYGENASE INHIBITORS WITH THIAZOL-4-ONE SCAFFOLD

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Lipoxygenases (LO) play a pivotal role in the metabolism of arachidonic acid (AA) with their catabolic pathway into leukotrienes (LT). One of the most important enzymes here is the 5-lipoxygenase (5-LO). Due to the key function of LTs on inflammation[1] and cancer[2], the inhibition of their effects moved into the focus of several areas of research. At this moment, there are only two drugs admitted for therapy: Montelukast (Singulair®), an antagonist of $CysLT_1$ receptors, and Zileuton (Zyflo®), a direct inhibitor of the 5-LO. Both drugs are admitted for the therapy of asthma bronchiale. Because of further medical need for alternative treatment options, the development of new direct inhibitors of the 5-LO is of permanent interest. By the inhibition of 5-LO the LT-formation is prevented on an early stage. Based on recently published lead stuctures[3] we designed and synthesized new derivatives of compounds with a central thiazol-4-one-moiety as direct inhibitors of 5-LO. The thiazolone scaffold is substituted on its 2- and 5-positions as eastern and western part, respectively. The compounds could be prepared in an one-pot-synthesis procedure from *p*-methoxybenzaldehyde, thioglycolic acid and differently substituted benzonitriles[4]. In these derivatives the western and the central part remained constant. The eastern part has been varied with different electron-releasing and -withdrawing moieties as well as with small and larger substituents of different steric and lipophilic properties. The results of the activity assays using a cell-free 5-LO assay and on whole cells (PMNL cells)[3] showed highly comparable inhibitory potencies despite the large structural variations on the substituents. The IC_{50} values ranged from 0.09 to 0.65 µM in the cell-free assay and from 0.40 to 0.86 µM in the whole cell assay. It is concluded that large structural variations as substituents on the eastern part are accepted, but that they do not give great enhancement of inhibitory affinity at 5-LO.

Supported by LOEWE LiFF and LOEWE OSF funding grants.
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C137

pH-SENSITIVE CISPLATIN LIPOSOMES AS A TOOL FOR BYPASSING CHEMORESISTANCE IN OVARIAN CANCER CELLS

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Cisplatin is a well-established cytostatic agent in the therapy of ovarian carcinoma. However, the therapeutic application and benefit of ciplatin is often restricted by the development of chemoresistance.
A recent study reported on enhanced cisplatin uptake and cytotoxicity in resistant ovarian cancer cells by a liposomal formulation [1]. However, the intracellular release of the drug from the liposomes appeared to be a critical limitation. To further raise intracellular platinum levels, it is required to improve the mechanism of release. pH-sensitivity of liposomes by acid-labile bilayer composition is an already accepted approach [2].
The aim of this study was to prepare pH-sensitive cisplatin containing liposomes and to investigate their cytotoxicity in two sublines of ovarian cancer cells (A2780), sensitive and cisplatin-resistant ones.
pH-sensitive liposomes were prepared from a DOPE/Chems (7/3) lipid composition. pH-sensitivity of these liposomes was confirmed by fluorescent marker release in dependence on pH. In order to induce an endocytotic uptake of the liposomes by transferrin-receptors, holotransferrin was coupled onto the terminal ends of a PEG anchor (1 mol%).
The platinum uptake by both cell lines was analyzed by flameless AAS for different incubation times between 0.5 h and 24 h and related to total protein amounts. It was evident that platinum accumulated in both cell lines in a nearly similar extent, which exceeded the level of the free drug in the resistant cells clearly. However, the transferrin targeting was of minor importance for the drug uptake. The findings were correlated with cytotoxicity data based on the MTT-assay at different time points (24 h, 48 h, 72 h). The comparison of the pH-sensitive with conventional cisplatin liposomes or the free drug strongly underscores the potential of pH-sensitive liposomes to overcome platinum resistance.

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C138

GOLD(I) CARBENE COMPLEXES: A CLASS OF COMPOUNDS WITH A HIGH BIOLOGICAL POTENTIAL

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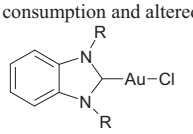
With the development of Auranofin the interest in the anticancer properties of gold complexes raised. However, remaining problems (such as the strong metabolization of Auranofin) did not allow a proper drug-design. Thioredoxin reductase (TrxR), an ubiquitary NADPH-dependent flavoenzyme, is nowadays considered as the main target for gold complexes. TrxR plays a central role in cell pathophysiology (proliferation, apoptosis, and metastasis) and is overexpressed in tumor cells. [1,2] Based on a molecular modelling study the synthesis of a series of disubstituted gold(I) carbene complexes and their intensive biological investigation was performed. The results indicated a strong activity on TrxR, selectivity against glutathione reductase (GR) and a remarkable stability against inactivation by glutathione. The target compounds **1a-4a** (Fig.1) demonstrated interesting cytotoxic properties, induced apoptosis, dysregulated the cell metabolism, enhanced the formation of reactive oxygen species (ROS), reduced the oxygen consumption and altered the cell morphology.



1a: R = methyl
2a: R = ethyl
3a: R = benzyl
4a: R = diphenylmethyl

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C139

CYTOTOXICITY AND BIOLOGICAL ACTIVITY OF RHODIUM(III) AND IRIDIUM(III) COMPLEXES

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The rhodium(III) and iridium(III) complexes of the type $[(C_5Me_5)MX(pp)]^+$ and $[MX_3(L)(pp)]$ with pp = phen, dpq, dppz, dppn and X = Cl, Br represent a new class of cytotoxic substances. Cytotoxicity and cellular uptake studies on the MCF-7 and HT-29 cell lines have also been employed to establish relationships between the structure and the activity of *mer- /fac-* $[MX_3(L)(pp)]$ complexes (with L=H_2O, CH_3OH, 1-methylimidazole, DMSO; X=Cl, Br and M=Rh, Ir) [1,2].

One goal of our ongoing studies is to improve the cell selectivity and identify lead substances by varying the ligands L and pp in complexes $[(\eta^5\text{-}C_5Me_5)RhL(pp)](CF_3SO_3)_n$. It has previously been shown that there are significant differences between the cytotoxicities of Pt(II) complexes with methylated 1,10-phenanthroline ligands [2]. We now report studies of the biological activity of $[(\eta^5\text{-}C_5Me_5)RhCl(pp)]CF_3SO_3$ complexes containing methylated phenanthroline ligands and other substituted polypyridyl ligands. Measurements of the LDH release for lymphoma (BJAB) cells after 1h incubation with phen, 5,6-Me_2phen and dppz complexes demonstrated that unspecific necrosis is negligible. Specific cell death apoptosis via DNA fragmentation was detected for BJAB cells after 72 h.

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C140

SCHIFF BASE TRANSITION METAL COMPLEXES INDUCE STRONG ANTILEUKEMIA AND ANTILYMPHOMA EFFECTS

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Schiff base transition metal complexes exert various biological effects including antifungal, antibacterial and even antitumor activities. Therefore, they are becoming more and more attractive lead structures for the design of cytostatics with a mode of action that differs from that of the frequently administered anticancer agent cisplatin. Data on antileukemia and antilymphoma effects of Schiff base transition metal complexes, however, are limited.
The activity of salophene (compound 1; N,N´-bis(salicylidene)-1,2-phenylenediamine), its iron (II/III) and manganese (II/III) complexes as well as saldach (compound 2; rac-trans-N,N´-bis(salicylidene)-1,2-cyclohexanediamine) and its respective iron (II/III) complexes was evaluated against leukemia and non-Hodgkin's lymphoma cell lines.
The free ligands (complexes 1 and 2) induced in all cell lines, if at all, only marginal, concentration-dependent cell growth-inhibitory effects, and did not trigger Cu/Zn superoxide dismutase (Cu/Zn SOD) release (an indirect marker for oxidative stress) or induce apoptosis. The Schiff base transition metal complexes $[Fe^{II}(salophene)]$ and $[Fe^{III}(salophene)Cl]$ blocked cellular growth, caused a strong release of Cu/Zn SOD and induced apoptosis. In contrast, the manganese analogs $[Mn^{II}(salophene)]$ and $[Mn^{III}(salophene)OAc]$ inhibited cell growth, caused the programmed cell death only at higher concentrations and did not provoke release of Cu/Zn SOD in any of the cell lines. The ligand structure and regioisomery exerted a significant influence on the activity of the complexes. Methoxy-substituted iron (III) salophene compounds induced even stronger effects on the cell lines than their unsubstituted counter parts. However, weaker cell growth-inhibiting and cell death-promoting effects were observed when the salophene moiety of $[Fe^{II}(salophene)]$ and $[Fe^{III}(salophene)Cl]$ was replaced with saldach.
In conclusion, Schiff base transition metal complexes exert strong inhibitory activities on human leukemia and lymphoma cells.

C141

SYNTHESIS, CYTOTOXIC AND ANTIMICROBIAL ACTIVITIES OF HETEROCYCLIC TRANSITION METAL ION COMPLEXES

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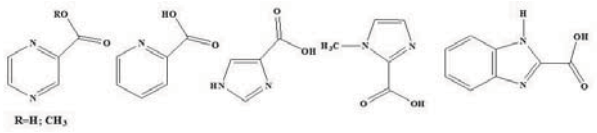
The goal of this project was to explore the biological activities of Cu(II), Co(II), Zn(II) and Pt(II) complexes bearing chelating heterocyclic carboxylic ligands. Transition metal complexes were synthesized with 2-pyrazinecarboxylic acid, methyl-2-pyrazinecarboxylic acid, 2-picolinic acid, 4-imidazole-carboxylic acid, benzimidazole-2-carboxylic acid and 1-methylimidazole-2-carboxylic acid ligands (Fig. 1). The structures of the complexes were established by the IR, ^{1}H-NMR, ^{195}Pt-NMR, elemental analysis and some by X-ray crystal analyses. In search for the most stable structure, the energies of the cis and trans isomers of the 4-imidazole-carboxylates have been studied by computational (DFT) methods.



Structures of the ligands.

The metal ion complexes were tested for their potential cytotoxic and antimicrobial activities. The cytotoxicity testing was done in a panel of human cancer cell lines on the 96 well microtiterplates using the crystal violet assay. The potency of compounds was estimated by the IC_{50} value in a panel of six cancer cell lines. Some platinum complexes showed promising cytotoxic activity. In the evaluation of antimicrobial activity the complexes were tested by using a modified agar diffusion method on five bacterial strains: *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *C. maltosa*. The evaluation of the activity was done by the measurement of the inhibition zones on the agar plates. Some of the Co (II) complexes gave inhibition zones of 19 mm or greater. The MIC values for the most active compounds have also been determined with the help of a microdilution test.

C142

DEVELOPMENT OF SIGMA-RECEPTOR BINDING HETEROCYCLIC DERIVATIVES WITH CYTOTOXIC ACTIVITY

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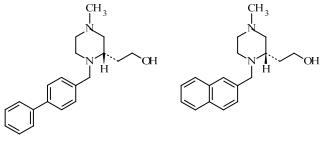
Sigma receptors are present in peripheral organs and in the central nervous system. They have also been found in many cancer cell lines. It is known that they are over-expressed in certain tumor cell lines and that synthetic ligands to this receptor could play an important role in cancer diagnosis and therapy. The aim of this project is to determine the cytotoxic activity of new sigma receptor ligands with conformationally constrained piperazines and their flexible analogues.



WMS 34-03 and WMS 34-04

The synthesized heterocycles were tested for their growth inhibitory activities in a panel of five human tumor cell lines by using a microtiter assay based on the crystal violet method for adherent cells. Growth inhibition of cells in suspension was measured with the MTT method. The potency was characterized by the GI_{50} value. WMS 34-03 and WMS 34-04 show promising cytotoxic activity with GI_{50} values around 10 µM. These substances are being investigated for their ability to induce apoptosis. Selected compounds will also be characterized in metabolism studies. In addition, we analyzed for the σ_1 receptor in RPMI 8226 cells with Western blot analysis. This human multiple myeloma cell line, which is described to express σ_1 receptors in high density, is being used to conduct receptor binding assays, the results of which will be presented.

C143

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SPLITOMICIN ANALOGS TARGETED AT Sirt1

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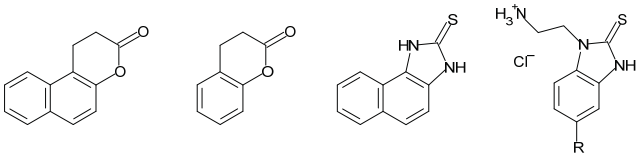
Small molecules interfering with posttranslational modification of histones are of interest as tools to study epigenetic regulation of gene transcription. Known post-translational modifications consist of protein phosphorylations, methylations, acetylations and deactylations. Specifically, drugs that interfere with histone deacetylation could be useful to induce differentiation, growth arrest as well as apoptotic cell death in tumor cells. In addition, the "histone code" code that is based on molecular processing of histones by certain enzymes complements the genetic code and thus enables novel strategies for therapeutic intervention. Different classes of histone deacetylases have been described as key players in this context. Deacetylases that incorporate a divalent Zn atom in the active site can be targeted with hydroxamic acid warheads. Another class of histone deacetylases is known as Sirtuins some of which (*S. cerevisiae* Sir2) are for example inhibited by the lactone splitomicin (**1**) leading to telomeric silencing in yeast. However, splitomicin is only a micromolar inhibitor of yeast Sir2 and does not inhibit human subtypes and the lactone is prone to hydrolytic ring opening. In preliminary SAR-studies, analogs of lead **1** lacking this hydrolytically labile ring were described as inactive while the naphthalene moiety could successfully be replaced by smaller aromatic rings such as benzene in the fragment-like dihydrocoumarin **2**. Here we report the synthesis and biological activity of a series of hydrolytically stable analogs of **1** and **2** such as compounds **3** and **4** with activity against Sirt1. These comparatively small compounds characterized by high ligand efficiency are used as a starting point toward the development of specific inhibitors of human Sirt1-like histone deacetylases.



1 **2** **3** **4**

C144

SYNTHESIS AND CYTOTOXICITY OF ADENINE ANALOGS AS FRAGMENTS AND LIGANDS FOR DRUG DESIGN STUDIES

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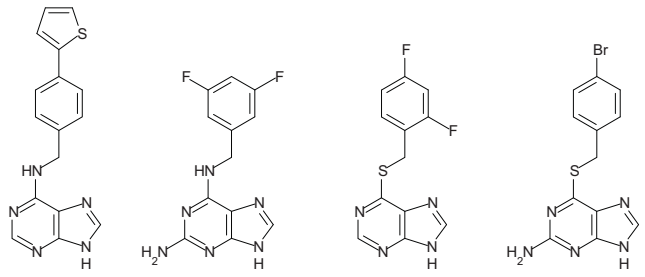
The hydrogen-bonding capacity and versatile coordination ability of adenine toward metal ions makes this fragment a privileged structure in medicinal chemistry. The former constitutes molecular recognition motifs crucial for correct DNA base-pairing and stabilization of double-helical structure, while the latter not only is manifested on charge dissipation but also influences pK_a of the exocyclic nitrogen. The five-membered imidazole ring harbored in the adenine framework makes this nucleobase also effective in biochemical catalysis, for instance in the ribosomal peptidyltransferase center. With this in mind, we have exploited additional adenine derived structures as fragment-like building blocks for bioactive compounds. To gain knowledge in the field of the versatile functional roles of the adenine framework, we generated decorated adenines with substituents on the C2 and/or C6, position leaving the four imino nitrogen atoms unaltered. Since naturally occurring nucleobases permit versatile metal ion coordination, which is usually invoked for metal ion-nucleic acid interactions, these novel derivatives hold potential as ligands for the construction of new platinum derived complexes for anticancer therapy. These novel fragments might well be useful in the construction of drug-like molecules and thus could be formed by metabolic processes. Herein we report the cytotoxicity of these molecules for the first time.



C145

SYNTHESIS OF NEW NICOTINAMIDE-ANALOGUES AS POTENTIAL SIRTUIN INHIBITORS

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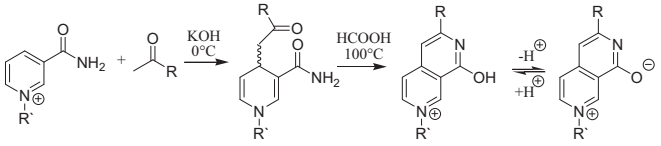
Sirtuins represent a specific NAD^+-dependent class of histone deacetylases (HDACs). By using NAD^+ as a cofactor, these enzymes cleave off the acetyl groups from the ε-amino group of lysines in histones and other proteins, e.g. p53, FOXO proteins, p300 or HIV tat. The human family of sirtuins consists of seven members, which are distributed to different cell compartments and involved in the regulation of various physiological processes like apoptosis, cell differentiation, metabolism, DNA recombination and HIV tat transactivation. Thus, sirtuin inhibitors are interesting potential drugs for drug discovery.[1]
Besides the deacetylated lysines, the products of the catalytic reaction are 2`-O-acetyl-adenosine diphosphate ribose and nicotinamide, which itself acts as a physiological sirtuin inhibitor. Thus, we chose nicotinamide as a lead structure for synthesis of new potential sirtuin inhibitors.

Synthesis of 2,7-Naphthyridones[2]
Quaternary N^1-Alkylnicotinamides were treated with an alkaline solution of methylketones. After a nucleophilic addition of the deprotonated methylketone compound to the pyridinium salts in the 4-position, the 2,7-naphthyridones were formed by dehydrogenation. As an oxidant formic acid was used. The highly fluorescent products were either isolated as a dipolar ion out of the aqueous layer or its hydrochloride out of an ethanolic phase.



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C146

LESTAURTINIB INHIBITS THE KINASE PRK1 IN VIVO

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Epigenetics is the study of changes in the protein expression caused by mechanisms other than the change of the underlying DNA sequence. These alterations in protein expression can be carried out by microRNA, DNA methylation and diverse histone modifications. In contrast to histone acetylation and methylation very little is known about histone phosphorylation.
By now several kinases are known to play an important role in the regulation of gene expression. For androgen receptor signalling, PKC betaI and PRK1 have been shown to have crucial functions in activating gene transcription.
Because of its activating role, PRK1 is considered to be a promising target for the treatment of prostate cancer.[1] Here we present a focussed library screening approach using known kinase inhibitors to identify PRK inhibitors. We identified the clinical candidate Lestaurtinib (see Figure 1) as a potent inhibitor of the epigenetic kinase PRK1.

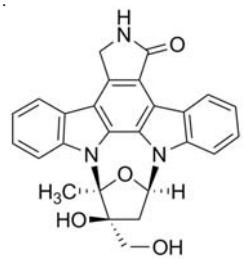

Figure 1: Chemical Structure of the PRK1-inhibitor Lestaurtinib

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C147

INVESTIGATION OF THE INFLUENCE OF THE POSITIONS OF THE PHENOLIC HYDROXYL GROUPS IN 2,2´-BISBENZIMIDAZOLES CONCERNING THE EFFECT ON FLUORESCENT PROPERTIES

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Breast cancer is one of the most frequent cancers in women. About 60 percent of this tumor entity are hormone dependent, which means they contain estrogen receptors and require estradiol for growing. Therefore the differentiation between hormone dependent and hormone independent cancers plays an important role with regards to the individual therapy. Presently radioactive methods are used for diagnostic. In recent years promising fluorescent 1,1´-dialkyl-2,2´-bisbenz-imidazole derivates have been synthesised in our group. These compounds shall be used for visualising the estrogen receptor in a non-radioactive method. In addition the influence of the positions of the phenolic hydroxyl groups in the benzimidazol rings have been investigated concerning the effect on absorption and fluorescent properties. Furthermore compounds with a potential (anti)estrogenic effect have been developed.
All of these compounds meet the structural requirements to interact with estrogen receptors. Currently methods are set up to determine their binding quality to the estrogen receptors in whole cell assays and with isolated receptors. The (anti)estrogenic effect and the influence on the receptor density of these compounds are investigated in transactivation assays and western blots.

C148

SYNTHESIS AND INVESTIGATION ON THE MODE OF ACTION OF (4R,5S)/(4S,5R)-2,4,5-TRIARYL-4,5-DIHYDRO-1H-IMIDAZOLES

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Almost 60 percent of mammary carcinoma are hormone dependent. The tumor cells contain estrogen receptors and require estradiol for proliferation. Selective estrogen receptor modulators (SERMs) are frequently used in tumor therapy because they have antiestrogenic effects in breast tissue but estrogenic effects in bones and the cardiovascular system.
In recent years in our group were a lot of imidazole-derivatives synthesised which meet the structural requirement for interactions with estrogen receptors. The compounds are currently investigated for their effects in human hormone (in)dependent tumor cells. Compounds with aromatic hydroxyl groups reveal interaction with the estrogen receptor. In this context compounds with amino groups instead will be synthesised and analyzes in termes of estrogenic activity.
It has been shown that derivatives with lipophilic substituents lack estrogenic activity but cause unlike hydroxylated compounds cytotoxic effects on testet tumor cells. Whether the cytotoxicity is caused by necrotic or apoptotic processes has to be resolved in LDH-release- and caspase-3-assays.
Further compounds have to be synthesised for performing SAR studies which should reveal the essential structural elements for cytotoxic and (anti)estrogenic effects. For this purpose we are now establishing new methods of synthesis using a microwave.

C149

IS THE CCN1 PATHWAY RELEVANT FOR INTEGRIN FUNCTION IN MELANOMA METASTASIS AND INTERFERENCE WITH HEPARIN?

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The integrin VLA-4 (very late activation antigen-4) on the human melanoma cell line MV3 is crucial for cell adhesion in course of hematogenous metastasis. With respect to therapeutic interference in metastasis, heparin was found to inhibit MV3 cell adhesion to VCAM-1 *in vitro*. Recent studies refer to structural requirements of heparin to reduce MV3 binding, but could not finally confirm the heparin binding to VLA-4, since several factors influence integrin signaling and activation. Recently, the activation of other integrins than VLA-4 on tumor cells was shown by binding to secreted cysteine-rich proteine 61 (Cyr61/CCN1) as a kind of autocrine stimulation. Since Cyr61 has binding ability to heparin, an indirect activity of heparin on VLA-4 via Cyr 61 can be assumed.
To confirm direct binding of heparin to VLA-4, SAW biosensor studies were performed using a series of modified heparins and a VLA-4 containing MV3 membrane preparation providing kinetic binding data. To further focus on the heparin binding pathway and Cyr61, the effects of exogenous added Cyr61 and the downregulation of Cyr61 in MV3 melanoma cells by shRNA technology were analysed.
The kinetic binding data suggest a direct interaction between heparin and VLA-4. Binding affinities of fractionated heparin in the low micromolar range were attenuated by N-acetylation or size fractionation of heparin. Other modifications, such as partial desulfation or ring opening of the saccharides less affected the affinity or slightly increased binding. Preliminary data suggest a reduced binding of Cyr61-knockdown cells. The hypothesis of heparin interference on this pathway can be assumed and will be the matter of further investigations.
However, our data provide evidence for a direct interference of heparin with VLA-4 mediated melanoma cell binding. This sheds light on the use of heparin in antimetastatic approaches.

C150

SYNTHESIS OF PHARMACOLOGICAL INHIBITORS OF HSF-1/HSP70 FOR THE TREATMENT OF MULTIPLE MYELOM

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Since multiple myeloma (MM) is still a remediless disease, the development of novel drugs is urgently needed. The observation that heat shock proteins (HSP) are overexpressed in MM cells and that their genetically deactivation induces apoptosis of the cancer cells reveal HSPs as promising targets.[1] It was furthermore ascertained that knocking down HSF-1, which is an important transcriptional activator of HSP70 expression, similarly induces apoptosis.[2] However, efficient and selective inhibitors of HSF-1 or HSP70 are still not available.
Derived from the known inhibitor emetine, several substituted quinolines and isoquinolines were synthesized and screened for their ability to inhibit the HSF-mediated HSP70 induction. The first results revealed the tetrahydroisoquinole skeleton as a promising lead structure. Therefore several highly diverse tetrahydroisoquinolines have been synthesized to prove this concept and gain access to structure-activity relationship analysis.

Literature:
[1] Chatterjee, M.; Jain, S.; Stuehmer, T.; Andrulis, M.; Ungethuem, U.; Kuban, R.-J.; Lorentz, H.; Bommert, K.; Topp, M.; Kraemer, D.; Mueller-Hermelink, H. K.; Einsele, H.; Greiner, A.; Bargou, R. C., *Blood* **2007,** *109* (2), 720-728.
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C151

INDENO[1,2-B]INDOLE DERIVATIVES ARE COMPETITIVE INHIBITORS OF THE HUMAN PROTEIN KINASE CK2

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Protein kinases in general are thoroughly investigated drug targets and important targets for therapeutic intervention. Protein kinase CK2 is a ubiquitous serine/threonine kinase capable to phosphorylate a wide array of substrates in vitro. To date well over 300 potential physiological targets of CK2 have been identified, but it seems unlikely that the enzyme plays any role in the in vivo phosphorylation of casein, the protein from which its name originally derived. Beyond the importance of CK2 in the context of cell survival and cell proliferation, there is a large body of evidence that CK2 is involved in neoplastic transformation and cancer. In a number of different cancers, including those of the prostate, mammary gland, lung and others, abnormal high levels of CK2 have been observed. This suggests CK2 to be an attractive and promising target for anti-neoplastic therapeutics, but despite the growing evidence on CK2 participation in malignant transformation and cancer, only few inhibitors of the enzyme are available. In the present study, partially hydrogenated indeno[1,2-b]indoles were synthesized using a novel established protocol and after dehydrogenation, followed by oxidation a series of compounds were obtained [1] and tested for CK2 inhibition by the use of the recombinant human enzyme expressed in E. coli. Competitive inhibition was exemplarily demonstrated and new inhibitors with IC_{50} values in the nanomolar range were identified.

[1] Hemmerling, H J, Götz, C, Jose, J (2008) Substituted indeno[1,2-b]indole derivatives as novel inhibitors of protein kinase CK2 and their use as tumour therapeutic agents, cytostatics and diagnostic aids. *Patent* WO2008040547

C152

BENZOFURANONE TF INHIBITS PROTEIN KINASE CK2 AND SHOWS PRO-APOPTOTIC EFFECTS IN PROSTATE CANCER CELLS

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Increased cellular CK2 activity was shown to contribute to the development and the maintenance of cancer. It is directly correlated to the dysregulation of many signal transduction pathways. Furthermore, there is strong evidence that elevated CK2 activity suppresses apoptosis and enhances cell proliferation. As a consequence the cell is predisposed to develop and maintain neoplastic diseases. Therefore, CK2 is considered a promising target for the development of anti-cancer drugs that reduce CK2 activity to a non-pathogenic level.
To identify potential drug candidates, we developed a novel non-radiometric activity assay based on capillary electrophoresis using the recombinantly expressed human holoenzyme $CK2\alpha_2\beta_2$ [1]. This assay allowed a library screening for CK2-inhibiting compounds. By this means, we could identify the compound TF as a novel potent inhibitor with an IC_{50} value of 30 nM. Treatment of the prostate carcinoma cell line LNCaP with TF led to an inhibition of endogenous CK2 activity and a decrease of cell viability measured by an MTT assay. Furthermore, we could demonstrate that incubation with TF induced apoptosis in these cancer cells. These effects were nearly identical to those obtained with the known inhibitor TBB used as a control. First results of a selectivity profiling with a TF-concentration of 10 µM and 63 human protein kinases confirmed that TF is indeed an inhibitor with selectivity for CK2.

[1] Gratz A, Götz C, Jose J (2010) A CE-based assay for human protein kinase CK2 activity measurement and inhibitor screening. Electrophoresis 31:634-40.

C153

REPRESSION OF THE PROTO-ONCOGENE Pim-1 BY miR-33a

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MiRNAs are small ncRNAs that act as cellular regulators of development, proliferation, differentiation, apoptosis and stress response. So far, miRNAs are known to modulate gene expression in most species, including *Homo sapiens*.
In treatment of cancer, a miRNA-based strategy using miRNA mimics or anti-microRNAs (AntimiRs) generates the ability to regulate a whole network of co-interacting pathways. This could give the opportunity to modulate bio-pathologic features and disease outcomes. [1]

Here we present the proto-oncogenic kinase Pim-1 to be the first cell cycle relevant target of miRNA 33a. We found that the erythroleukemia cell line K562 and the colon carcinoma cell line LS174T, which have high and moderate Pim-1 mRNA and protein levels, express low cellular miR-33a levels compared to other miRNAs like the oncogenic miR-17-5p or miR-20a.
Within its 3'-UTR the Pim-1 kinase harbours a highly conserved binding site for miR-33a. We found that transfection of a miR-33a mimic leads to specific downregulation of Pim-1 at the mRNA and protein levels. Seed mutagenesis of the miR-33a target sequence in the 3'-UTR of Pim-1 demonstrated specificity of miR-33a dependent regulation using a luciferase reporter assay. Moreover, transfection of the related miR-33b mimic has no effect on Pim-1 expression. The efficacy of the miR-33a dependent knockdown is not as strong but as persistent as obtained with selected Pim-1 specific siRNAs. RNAi-dependent downregulation of Pim-1 inhibited proliferation in K562 and LS174T cells by arresting cells in G2/M phase of the cell cycle. Another cell cycle regulator, the kinase Cdk6, is not regulated by a miR-33a mimic although its priority to be targeted by miR-33a is ranked at higher position using the prediction tool TargetScan 5.1.

[1]: Iorio MV, Croce CM (2009) MicroRNAs in Cancer: Small Molecules With a Huge Impact. *J Clin Oncol* **27:** 5848-56

C154

SYNTHESIS AND BIOLOGICAL EVALUATION OF SIMPLE PLATENSIMYCIN ANALOGUES

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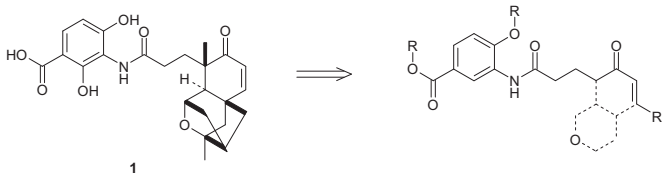
The natural occurring antibiotic platensimycin (**1**) is an interesting lead structure for developing new antiinfectives due to its new mechanism of action, the inhibition of bacterial fatty acid biosynthesis.
Since the total synthesis of platensimycin can be accomplished only by a multistep protocol, we intended to prepare simple analogues of this natural product, which still contain the functional groups that have previously been identified as being essential for binding to the target enzyme in fatty acid biosynthesis FabF/B (β-ketoacyl-(acyl-carrier-protein (ACP) synthase I/II).

Essential residues for binding of platensimycin are the acylamino hydroxybenzoic acid structure, and the keto group and the cyclic ether in the aliphatic region.

So our synthesis of the simple analogues focussed on conserving these elements. The synthesized analogues of platensimycin showed interesting antibiotic activities in an agar diffusion assay comparable to common antibiotics.



1

C155

SYNTHESIS AND BIOLOGICAL EVALUATION OF BERBERINE DERIVATIVES

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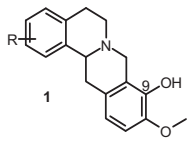
Isoquinoline alkaloids are distinguished by a bundle of activities, however all of them weak: antibacterial, antitumoral, anti-inflammatory, and antileishmanial [1-4]. This renders them ideal leads for a SOSA approach (Selective optimization of side activities, [5]).
We present the synthesis of isoquinolines with non-natural substitution pattern and test results for specific efficacy against bacteria, fungi, and human leukemic HL-60 cells.
Derivatives with a hydroxy group in position 9 (protoberberines of general formula, **1**) were found to have the largest effect against the fungal species tested (Aspergillus niger, Hyphopichia burtonii), while the quaternary congener berberine exhibited only marginal activity. We suppose the protoberberines to inhibit 24-sterolmethyltransferase (24-SMT) [6]. All compounds were weakly or not toxic against HL-60 cells.



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C156

HAEMAGGLUTININ CLEAVING PROTEASES – POSSIBLE TAGETS TO TREAT INFLUENZA INFEKTIONS

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Human Influenza viruses cause acute infection of the respiratory tract that affects millions of people during seasonal outbreaks every year. Recently, the outbreak of a swine origin H1N1 influenza virus for which there is no or little immunity in humans exacerbates the situation. Currently, only two drugs targeting the viral neuraminidase and the M2-channel protein inhibitor amantadine are approved for the treatment of influenza in the EU. Infectivity of influenza viruses strongly depends on correct cleavage of the haemagglutinin precursor HA0 by host cell proteases. All human pathogen HA precursors have a single arginine at the cleavage site. Recently, two membrane-bound trypsin-like serine proteases, TMPRSS2 (transmembrane protease serine S1 member 2; epitheliasin) and HAT (human airway trypsin-like protease or TMPRSS11D), localized in human airway epithelium, were identified, which activate HA and support replication of the viruses. Meanwhile we could demonstrate that HAT is proteolytically active on the cell surface, whereas TMPRSS2 cleaves HA0 within the cell. Both proteases are potential targets for the treatment of influenza. In contrast to HAT there is no commercial source for TMPRSS2. Therefore, we started our work with the expression, purification and activation of TMPRSS2 including their enzyme kinetic characterization. Among a series of substrate-analogue peptide mimetics containing a 4-amidino-benzylamide as P1 residue we could identify first leads, which inhibit TMPRSS2 and HAT in the nanomolar range. Although some of these analogues had similar potency in enzyme kinetic studies, we observed significant differences in their efficacy to inhibit virus propagation in TMPRSS2 or HAT expressing cell lines.

C157

HIT VALIDATION FOR A FLUORIMETRIC SARS COV MAIN PROTEASE ASSAY

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Development of highly specific inhibitors for SARS CoV main protease which do not form a covalent bond with the protease and which do inhibit the protease in a competitive way is quite challenging. The determination of the potency and kinetic of potential inhibitors can be carried out by performing a fluorimetric enzymatic assay based on the principles of fluorescence resonance energy transfer (FRET). Mechanistically different effects other than inhibition, such as the inner filter effect or colloidal aggregation, can bias the measurement and may cause false positive results. Therefore a validated workflow is of utmost importance to prove the correct inhibitory potency. Simple methods to distinguish hits from false positives are proposed. Addition of Triton X-100 suppresses unspecific interactions between enzyme and inhibitor by forming colloidal aggregates. Moreover, with FRET assays the inner filter effect needs to be considered. It is caused amongst others by inhibitors which absorb light at the excitation or emission wavelength of the substrate and can be corrected by determining a correction factor dependent on the inhibitor concentration. Inhibitors binding covalently to the active site cysteine residue can be detected by the addition of dithiothreitol as an alternative thiol donor. If potential inhibitors still show activity after these tests, their kinetic characteristics need to be evaluated. Finally, the selectivity of the potential inhibitor should be checked against various different proteases. The aforementioned hit validation protocol will be exemplified with recently published SARS CoV M^{Pro} inhibitors.

C158

BISQUATERNARY NAPHTHALIMIDES – NOVEL ACTIVE COMPOUNDS AGAINST PLASMODIA, TRYPANOSOMA AND STAPHYLOCCOCI

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Malaria is the world's most prevalent tropical disease. The currently available drugs are showing increasing resistance and they are partially too expensive for of Africa. The situation of Trypanosoma ssp. is comparable. The bisquaternary bisnaphthalimides are a versatile class of compounds being active against *Plasmodium falciparum*[1], *Trypanosoma brucei brucei*[2] and *Staphylococcus* strains[3] in the low micromolar and nanomolar range of concentration. The qualitative analysis of the structure-activity relationships revealed the importance of a long methylene middle chain of at least 8 methylene groups between the two bisquaternary naphthalimides or a monoquaternary naphthalimide consisting of a long alkyl chain attached to the positively charged nitrogen atom (for Plasmodia and Trypanosoma). As a singularity one bisnaphthalimide exhibits a high activity against several strains of *Staphylococcus,* especially multi-resistant strains. Radioactive labeling, surface plasmon resonance and gel retardation experiments revealed a direct and unspecific binding to the DNA of Staphylococci. The cytotoxicity of these compounds studied here was evaluated and found to be very low. The structure activity relationships of cytotoxicity on the one hand and activity against Plasmodia, Trypanosoma and Staphyloccoci on the other hand are different. The mode of action in Plasmodia and Trypanosoma remains still to be elucidated.

[1] Holzgrabe, U.; *Antitrypanosomal activity of quarternary napthalimide derivatives;* Bioorg. Med. Chem. Lett. 2007, 17, 1590-1593
[2] Tischer, M.; *The Bisnaphthalimides as New Active Lead Compounds against Plasmodium falciparum;* Bioorg. Med Chem 2010, 18, 2987-3352
[3] Menzel, T. M.; *The novel bisquaternary bisnaphthalimide MT02 and its antibacterial mode of action against Stapylococcus aureus;* Antimicrob. Agents Chemother. 2010, [submitted]

C159

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP OF NOVEL QUINOLONE-TYPE COMPOUNDS AGAINST *TRYPANOSOMA BRUCEI*

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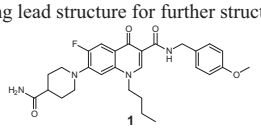
Human african trypanosomiasis (HAT) is caused by infection with one of two parasites, which differ in their geographical range and etiopathology. While *T.b. gambiense* HAT is primarily a human chronic disease and occurs in Western and Central Africa, *T.b. rhodesiense* HAT is primarily zoonotic with a huge animal reservoir and causes the acute form of sleeping sickness in Eastern and Southern Africa. Current estimations show that in the prevalence sub-Saharan area about 60 million people are at risk and 50000 – 70000 cases occur annually[1]. Both forms of HAT show two clinical stages, whereas the first corresponds to the multiplication of the parasite in the blood and lymphatic system. After crossing the blood-brain barrier the trypanosoma attack the central nervous system and neurological symptomes appear. Without medical treatment, coma and finally death results. Due to a low number of available drugs which suffer from severe side effects, new and easily accessible active compounds are urgently needed. Therefore we synthesized a quinolone-type library and tested them against *Trypanosoma brucei*. The 4-oxo-quinolone skeletons are build up using the Gould-Jacobs procedure with subsequent installation of the piperidine residue at C7 by nucleophilic aromatic substitution. The final step includes the amidation at C3 with different benzyl- and phenethylamines. The biological evaluation shows that the 4-oxo-quinolone-3-carboxylates are inactive. The essential increase in activity is obtained by amidation at C3. Moreover the piperidine residue at C7 and longer alkyl chains at N1 lead to further enhancement. Compound **1**, which exhibits an IC_{50} value of 0.78 µM, represents a promising lead structure for further structural modification.



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[1] E. M. Fèvre, B. v. Wissmann, S. C. Welburn, P. Lutumba, The Burden of Human African Trypanosomiasis, *PloS. Negl. Trop. Dis.*, **2008**, 2 (12).

C160

THE IMPORTANCE OF INHIBITING INFECTIVITY PROTEIN MIP FOR THE TREATMENT OF LEGIONELLOSIS

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Legionella pneumophila is a Gram-negative aerobic pathogen causing two distinct forms of Legionellosis: Legionnaires' disease, a severe pneumonia and Pontiac fever, a milder respiratory disease with symptoms resembling acute influenza. To date there is no efficient treatment for the severely progressing form with mortality rates of up to 20 %. [1] The bacteria occur in freshwater and are transmitted into human lungs i.e. alveolar macrophages [2] by inhaling *Legionella*-containing aerosols which can result from air condition systems, room air humidifiers, whirlpools or showers. In order to affect alveolar macrophages the bacteria have to cross epithelial cells and the extracellular matrix (ECM) of the lung tissue. This transmigration process is enabled by the macrophage-infectivity potentiator (Mip) protein which is arranged on the surface of the bacterium. Mip shows a peptidyl prolyl cis/tans isomerase activity and binds to collagen IV which is the prevalent collagen in the human lung. [3] In combination with another serine protease, Mip degrades ECM proteins and thereby allows the bacteria to cross the barrier. Hence, inhibiting Mip would hinder *Legionella* intrusion of macrophages and attenuate the infection.
Since no small-molecule inhibitor of Mip has been reported so far, the development of novel compounds against this target by means of computer-aided design, subsequent syntheses of selected molecules and their biological screening is the aim of this work. First pipecolic acid-type compounds have been already identified as "inhibitors" of Mip.

[1] Ceymann *et al: BMC Structural Biology* **2008**, 8: 17

[2] Steinert *et al: FEMS Microbiology Reviews* **2002**, 26: 149-162

[3] Gelse *et al: Advanced Drug Delivery Reviews* **2003**, 55:1531-1546

C161

LOW MOLECULAR CHROMONE-BASED COMPOUNDS AS POTENTIAL DRUGS AGAINST *MYCOBACTERIUM TUBERCULOSIS*

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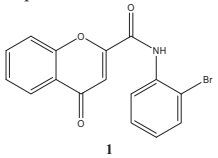
According to WHO, one third of the world′s population is infected with TB and about 50 million people suffer from the drug-resistant form. Of particular concern is the development of multi-drug-resistant forms of the disease (MDR-TB), defined as forms resistant to two or more of the front line anti-TB agents[1].

Hence, drug resistance in *Mycobacteria* has become a serious medical problem. Thus, there is an urgent need of new drug candidates displaying selective mode of action. One of the well described selective targets in *Mycobacteria* is the type II fatty acid synthase (FAS) system, which differs significantly from the fatty acid biosynthesis pathway in eukaryotic cells. The key regulator of the FAS II enzyme system in *Mycobacterium tuberculosis* is the β-ketoacyl-acyl carrier protein (ACP) synthase (KasA), which mediates the elongation of unsaturated fatty acid chains. ACP Synthase is inhibited by two natural products: thiolactomycin and cerulenin. Crystallographic data of the complex of KasA and thiolactomycin have been obtained recently[2] and were used for a virtual screening of the modified ZINC database of ca. 2.9 million compounds.



1

Among the structures found by virtual screening, chromone-moiety containing compound **1** was selected as a model structure of our study. Compound **1** displaying the inhibition activity on KasA in low micromolar range (K_d 26 ± 7 µM) can be regarded as a hit in the early enzyme assay. A set of related compounds was synthetized and its KasA inhibitory activity evaluated.

[1] TAACF Website: http://taacf.org/about-TB-background.htm
[2] Luckner, S.R. *et al*., Structure 2009, 17 (7), 1004-13.

C162

DEVELOPMENT OF NOVEL INHIBITORS OF KASA, A TARGET OF *MYCOBACTERIUM TUBERCULOSIS*

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Fatty acid biosynthesis, the first step in membrane lipid biogenesis, is catalyzed in bacteria by a series of proteins which is termed the type II fatty acid synthase (FAS) system. As the difference to the multifunctional type I synthase found in eucaryotes is significant, there is a possibility to design inhibitors of the FAS II system as effective and selective antibiotics against mycobacteria.
The enzyme of interest in our work is the β-keto-acyl ACP synthase (KasA), an elongating enzyme in the FAS II system of *Mycobacterium tuberculosis*. The recently solved crystal structure of KasA in complex with the well-known inhibitor thiolactomycin (TLM)[†] provides important information about the binding pocket, essential protein-ligand interactions and the mechanism of inhibition.
To identify novel lead structures a set of compounds has been retrieved by virtual screening of databases of commercially available compounds with a pharmacophore model based on the TLM binding mode. Subsequently, these compounds were docked into the KasA binding pocket to inspect the predicted binding modes, and the chemical accessibility of possible modifications was checked. Compounds with time-consuming synthesis were purchased, while more readily accessible substances from different chemical classes were synthesized and varied by altering the substitution pattern. Experimental testing of their inhibitory activity is currently in progress: The inhibitor-induced decrease of the intrinsic fluorescence of KasA is measured in a time-dependent manner in order to calculate K_d values.

References:
[†]Luckner, S. R. *et al*., Structure 2009, 17 (7), 1004-13.

C163

SYNTHESIS, MOLECULAR MODELLING AND PHARMACOLOGY OF DUAL HISTAMINE H_1/H_4-ANTAGONISTS

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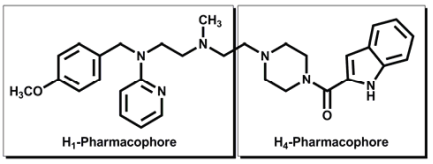
Indole and benzimidazole piperazine carboxamides are known to be potent histamine H_4-receptor antagonists [1]. The histamine H_4-receptor is discussed to be involved in inflammation and regulation of the immune system [2], whereas Histamine H_1-receptor antagonists like mepyramine, diphenhydramine and desloratidine are used for treatment of allergic diseases. New studies show that combined application of H_1- and H_4-antagonists in the acute murine asthma model exhibits synergistic inhibitory effects on eosinophil accumulation in the bronchoalveolar lavage fluid [3]. Thus, the design of dual H_1/H_4R-antagonists may result in a new class of drugs for therapy of type-I allergic diseases, like rhinitis and conjunctivitis. Therefore, we developed combined ligands with one H_1- and one H_4-pharmacophore, coupled by an alkyl spacer, as exemplary shown below.



Competition binding assays were used to determine affinity of the new compounds at hH_1R and hH_4R. Most of the compounds showed affinity to both, hH_1R and hH_4R. However, in dependence of structure, large differences in affinities were observed. Molecular modelling studies were used to explain these differences on a molecular level.

References:

[1] Venable et al. *J. Med. Chem.* **2005**, *48*, 8289-8298.
[2] Thurmond et al. *Nature Reviews Drug Discovery*, **2008**, *7*, 41-53.
[3] Deml et al. *Mol. Pharmacol.* **2009**, *76*, 1019-1030.

C164

MOLECULAR MODELING STUDIES ON THE FLUOROPHILIC PROPERTIES OF THE S^2 POCKET OF CATHEPSIN B

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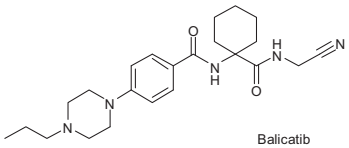
Cysteine proteases belonging to the papain-like subfamily are involved in important physiological processes such as antigen presentation, bone remodeling and apoptosis. Upregulation of these enzymes is implicated in autoimmune diseases, osteoporosis and cancer.[1-3] Cathepsin B is a cysteine protease involved in tumor invasion and metastasis.[3] It is also thought to play a role in the processing of the β-amyloid precursor protein.[4] Therefore, there is a considerable research focus on the design of new potent and selective inhibitors of cathepsin B.[2] In this regard, molecular modeling studies can be used to get insights into the properties of the enzyme active site and the binding mode of known inhibitors.
We will present our molecular modeling studies on the binding mode of chiral dipeptide-derived inhibitors[5] with structural similarity to the cathepsin K inhibitor balicatib.



Balicatib

The compounds possess a β,β-difluorinated cycloaliphatic moiety at the P^2position. Our results provide information on the interaction of the fluorinated face of the inhibitors with the S^2 pocket of the enzyme and let us conclude fluorophilic properties of the S^2 pocket of cathepsin B. Such information can be used for the design of further potent and selective inhibitors.

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C165

SYNTHESIS OF AMIDINES AS POTENT DDAH-1 INHIBITORS AND THEIR SELECTIVITY OVER ARGINASE AND NOS

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All three isoforms of NO-synthases (NOSs) are physiologically inhibited by endogenous N^{ω}-methylated L-arginines. These compounds are degraded by dimethylarginine dimethylaminohydrolase (DDAH) to L-citrulline and (di)methylamine. Thus, Inhibition of DDAH activity would lead to increasing N^{ω}-methylated L-arginine (NMMA, ADMA) levels, which could be another mechanism to indirectly affect nitric oxide (NO) formation.

To date, three different classes of potent inhibitors are known: (1) pentafluorophenyl sulfonates, which are also inhibitors for the arginine deiminase;[1] (2) the most potent inhibitors are based on indolylthiobarbituric acid,[2] but their activity seems to be restricted to bacterial DDAH; and (3) L-arginine analogs whose best representatives are N^{ω}-(2-methoxyethyl)-L-arginine and N^{δ}-(1-iminobut-3-enyl)-L-ornithine.[3,4]

Our approach was the development of amidine based L-arginine analogues as new inhibitors of DDAH-1 with selectivity over other enzymes especially NOS and arginase. These compounds were obtained by using Pinner synthesis.

Additionally, we developed an AFMoC 3D QSAR model based on experimental pKi values of known hDDAH-1 inhibitors.[5] It will be used for further structure based optimization of the underlying compound classes.

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C166

THE SEARCH FOR THE RIGHT POSE – A STRAIGHTFORWARD WORKFLOW FOR GSK-3 INHIBITORS

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Binding mode prediction is a challenging problem in drug design. Because of the huge number of different docking programs available today, the choice of the most appropriate is difficult and many evaluations have been published.[1] The focus was often on the enrichment in virtual screening protocols on the one hand. On the other hand approaches combining molecular dynamics (MD) with docking[2] provide valuable results in predicting binding modes. As MD-simulations are computationally expensive, moreover challenging in their interpretation and may also introduce significant error and noise in docking results,[3] we developed a straightforward workflow based on the combination of different docking programs, rescoring and complex minimization to identify native-like poses of protein kinase inhibitors.

In a cross docking experiment of GSK-3β complexes, results obtained with the docking programs AutoDock[4], FlexX[5] and Fred[6] were merged to account for different strengths and weaknesses of the respective programs. All poses were rescored with drugscoreX[7] and a rmsd-based clustering was performed. Afterwards a complex minimization was accomplished with Szybki[8] and the resulting complexes were again rescored. The workflow is described and its usefulness and limitations are discussed. It proofs valuable in identifying native-like poses of GSK-3β inhibitors and may therefore help identifying the correct binding mode of other protein kinase inhibitors without performing MD-simulations to account for receptor flexibility.

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C167

BINDING MODE PREDICTION OF *PF*GSK-3 INHIBITORS WITH A THIENO[2,3-*b*]PYRIDINE SCAFFOLD

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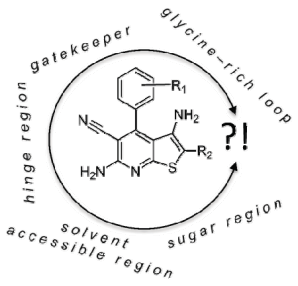
Even if the WHO states that the actual development gives cause for cautious optimism, malaria still remains a global emergency with estimated 243 million cases and 863 000 deaths in 2008 worldwide.[1] Emerging development of resistance even against the WHO-recommended first-line therapy[2] emphasizes the urgent need for new therapeutic concepts. Glycogen synthase kinase-3 of the malaria pathogen *Plasmodium falciparum* (*Pf*GSK-3) was suggested as a potential target for antimalarial drugs in 2004[3] and a series of compounds with selectivity over the human analogue of *Pf*GSK-3 was synthesized recently as part of a thesis.[4]



A crucial point in the further optimization will be the knowledge of the binding mode of the respective inhibitors. In the presented approach we used an extensive workflow for binding mode prediction to identify the most probable binding mode. The workflow combines docking into homology models of *Pf*GSK-3[5, 6] with different docking programs, rescoring and complex minimization.[7] The results will be presented here, they may help in understanding the structure activity relationships of existing inhibitors and give new impulses for further structural variations.

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C168

THREE STEPS AHEAD? A COMPARISON OF STRUCTURE-BASED AND LIGAND-BASED VIRTUAL SCREENING METHODS

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Virtual Screening has become a standard technique in medicinal chemistry to screen huge purchasable or virtual compound libraries *in silico* for molecules showing activity at a certain biological target. Thus Virtual Screening affords the reduction of compound sets from millions to a few hundred substances, which can be tested with available biological assay capacities.

As the amount of crystallographic data of proteins and co-crystallised inhibitors increases every year, structure based information supplies the medicinal chemist with striking information about a ligand binding to its target and may highlight the essential requirements a ligand needs for being an active molecule. If ligand–receptor complex data are not available, working on a 3D crystal structure only, or, working on ligand data of known active substances are two promising alternatives.

Here, three completely different virtual screening methods falling in three different categories are compared: LigandScout[1] a pharmacophore screening method that uses ligand-receptor complexes as input, FRED[2] a docking tool that uses receptor information only (i.e no co-crystallised ligand information is needed), and finally ROCS[3] that solely requires ligand information.

The performance is evaluated in a retrospective screening on the FieldScreen[4] dataset outlining strengths and weaknesses of each method for the scrutinized targets. This knowledge will help to design future prospective studies.

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C169

AZOLE DERIVATIVES AS HISTAMINE H_3 RECEPTOR ANTAGONISTS

Walter, M.[1], Isensee, K.[1], von Coburg, Y.[1], Kottke, T.[1], Ligneau, X.[2], Camelin, J.-C.[2], Schwartz, J.-C.[2], Stark, H.[1]
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Biogenic amines, such as histamine, play important roles as chemical mediators in human physiology. Histamine mediates numerous central and peripheral effects *via* four known G protein-coupled receptor subtypes (hH$_1$R-hH$_4$R). Human histamine H_3 receptors (hH$_3$R) are acting as autoreceptors on synthesis and liberation of histamine as well as heteroreceptors on modulating the release of several other neurotransmitters (e.g., acetylcholine, dopamine, glutamate, noradrenalin, serotonin, GABA). Due to a distinct receptor expression pattern in the central nervous system (CNS) and their involvement in several neuronal functions, e.g., vigilance, attention and learning, the hH$_3$R are attractive targets for the treatment of CNS disorders like schizophrenia, epilepsy, depression, Alzheimer's disease, Parkinson's disease and sleep disorders.[1]
Antagonist H$_3$R ligands follow a general pharmacophore blueprint, containing a basic moiety, mostly a tertiary amine, linked by a spacer to a central core substituted by a variety of structural elements providing different physicochemical properties.[2] In this investigation we focused on the variability of the core region by introducing polar heterocycles containing one to three nitrogen atoms (azoles) with different connecting moieties to the spacer. A diversity of structural motifs is used as substituents to optimize the receptor binding properties, which ranging from low nanomolar concentration to complete loss of affinity.
We successfully introduced polar azole groups as new central core element in the hH$_3$R antagonist pharmacophore and could establish a new structural class of hH$_3$R antagonists/inverse agonists showing the possibilities and limitations in structural variations on this central core element.

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C170

SYNTHESIS OF A FLUORESCENT ALLOSTERIC MODULATOR OF MUSCARINIC RECEPTORS

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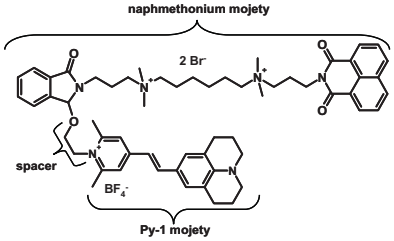
Allosteric modulators of muscarinic receptors, such as W84 or naphmethonium, are established potent agents capable of selectively binding to the M_2-subtype of the acetylcholine receptors M_1-M_5. They bind to a topographically different site than classical orthosteric ligands and are able to modulate the efficiency of orthosteric ligands by influencing both dissociation and association.[1] The potency of allosteric modulators is determined indirectly by measurement of the inhibition of the dissociation of the orthosteric ligand *N*-methyl-scopolamine (NMS) or directly by use of a radiolabelled W84 derivative. Since fluorescent dye labelled allosteric modulators may help to directly characterize allosteric interactions and trace receptor trafficking by means of fluorescence correlation spectroscopy, the aim of this work was to synthesize a naphmethonium derivative which is suitable for a connection to the fluorescent dye Py-1. Therefore, several strategies to connect a primary amino group *via* an alkyl-spacer to naphmethonium were prosecuted. Finally, synthesis of a naphmethonium derivative *via* a chloride-intermediate was successful. Subsequent conversion of this compound with Py-1 which was synthesized according to the literature[2] yielded the fluorescent naphmethonium derivative.



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C171

BENZOTHIAZINONES – A NEW CLASS OF POTENT A_1 ADENOSINE RECEPTOR ANTAGONISTS

Schlenk, M., Paskaleva, M. , Iqbal, J. , Gäb, J. , Gütschow, M., Müller, C. E.
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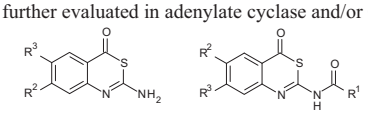
Several 3,1-benzothiazin-4-one derivatives originally synthesized as protease inhibitors were found to be potent antagonists at A_1 adenosine receptors (ARs). We subsequently evaluated a larger series of compounds **I** and **II** with broad variation of the substitution pattern. The synthesized compounds were investigated in radioligand binding studies at rat A_1, rat A_{2A}, human A_{2B} and human A_3 receptors. Selected compounds were additionally examined in radioligand binding studies at human A_1, human A_{2A}, and rat A_3 ARs in order to obtain information about potential species differences. The most potent compounds were further evaluated in adenylate cyclase and/or GTP shift assays.



I **II**

The investigated benzothiazinone derivatives proved to be antagonists at adenosine receptors. **Gü304** (**II**, R^1 = Ph, R^2 = H, R^3 = H) was the most potent compound with a K_i value of 7.70 nM at rat and 65.5 nM at human A_1 ARs. The compound also showed relatively high affinity for human A_3 ARs (K_i = 30.4 nM). Some derivatives exhibited relatively high affinity for A_{2B} receptors (K_i < 200 nM). In conclusion, we identified a novel chemical class of AR antagonists. The benzothiazinone scaffold was optimized towards high A_1 affinity. It bears the potential for development of potent and selective antagonists for other AR subtypes as well, namely for A_{2B} and A_3 ARs.

C172

MULTITARGET APPROACH TOWARDS ALZHEIMER'S DISEASE BASED ON DUO DERIVATIVES

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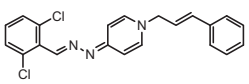
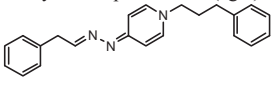
Based on DUO compounds, which are potent ditopic inhibitors of acetylcholin-esterase (AChE) [1], but too long for the catalytic gorge, new compounds have been developed. Using roughly only one part of the molecule led to permanently positively charged molecules, which are not able to pass the blood-brain barrier (BBB) anymore [2]. Therefore the pyridinium ring was replaced by a piperidine ring whose nitrogen can be protonated. Modeling studies revealed that the loss of activity is due to the non-aromatic piperidine ring. Thus a "flat" pyridine hydrazone ring system was employed. These new lead compounds show satisfying inhibitory effects on AChE, as well as an activity on butylcholinesterase (BuChE) and seem to have inhibitory effects in ROS tests. Furthermore they inhibit the amyloid β fibril formation and lead to a disaggregation of preformed amyloid β fibrils [3]. Even though the activity of these compounds has to be improved, they clearly show, that it is possible to develop multitarget molecules. New modeling studies point out a raised inhibitory effect on AChE elongating the hydrazone part or extending the conjugated system. Derivatives according to the modeling studies revealed a better inhibitory effect when the hydrazone part was elongated (eg **1**), while extending the conjugated system shows only low improvements (eg **2**).



Eg. **1** (IC50 AChE 0.94±0.07µM)



Eg. **2** (IC50 AChE 0.063±0.009 µM)

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C173

CARBAMATES AS CNS-TARGETING BACLOFEN PRODRUGS: STUDIES WITH METHYL CARBAMATE

Mosad,S.[1], Eddiasty,I.[1], Nieß, R.[1], Abouzid, K.[1], Hanafi, R.[1], Abdel-Kader, R.[1], Spahn-Langguth, H.[1,2],
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Baclofen, a specific $GABA_B$-receptor agonist, has successfully been used for decades to treat spastic disorders. However, only intrathecal dosing provides direct biophase access and yields effective CNS-levels, as indicated by the respective cerebrospinal fluid concentrations. This implies that baclofen does not sufficiently permeate through the blood-brain-barrier.
Since ester prodrugs of baclofen are not readily cleaved in vivo and were found to be subject to exsorptive transport, alternative prodrug concepts were evaluated.
E.g., methyl 4-(*tert*-butoxycarbonyl amino) 3-(4chlorophenyl) butanoate (baclofen methyl carbamate) was synthesized and its solubility and log P were evaluated as bio-relevant parameters. Baclofen methyl carbamate was then tested vs. baclofen with respect to the obtainable target organ levels.
Baclofen HCl as well as the methyl carbamate were dosed intraperitoneally to rats (baclofen dose: 1.0 mg/kg, carbamate dose, equimolar for direct comparability). Blood and brain samples were collected and the analytes quantified via RP-HPLC on an octadecylsilane stationary phase (gradient elution with pH2.6 0.1mM phosphate buffer/methanol mixtures; UV-eluate monitoring, 220 nm).
It was found that the additional carbamate group in the baclofen methyl ester molecule had a significant influence, since the structural change was considerably decreasing the solubility in water and increasing the partitioning into lipophilic matrices. Maximum baclofen concentrations detected in brain tissue were almost 5-times higher after carbamate dosage than after administration of baclofen itself. As opposed to parent baclofen the carbamate showed a rapid distribution into tissues after i.p. dosage. In the blood compartment formation of baclofen was negligible. In the brain the prodrug was hydrolyzed to a remarkable extent, yet showing a delayed release profile for baclofen and reaching highest brain concentrations at a t_{max} of 3 hr. The possibility to simultaneously detect the respective ester via HPLC gave evidence that the methyl ester cleavage represents the first of two sequential metabolic steps regenerating baclofen in vivo. Hence, carbamate derivatives appear as very promising biolabile prodrugs for brain delivery of baclofen.

C174

PYRIDINYL IMIDAZOLE COMPOUNDS AS SELECTIVE JNK3 INHIBITORS

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The c-Jun terminal kinases (JNKs) are members of the mitogen activated protein (MAP) kinase family and regulate signal transduction in response to environmental stress. It is reported, that activation of JNK3 causes neurological damage and therefore JNK3 might be an interesting target for the treatment of neurological disorders.
Pyridinyl imidazoles are known as potent inhibitors of p38α, which is a closely related MAP kinase family member differing at the ATP-binding site in only one amino acid at the gatekeeper region (Thr 106 vs. Met 146). The hydrophobic region II, a solvent exposed surface close to the ATP-site, is however less conserved.
By introduction of carboxylic acid moieties, targeting the hydrophobic region II, we obtained very potent inhibitors of JNK3 with high selectivity over p38.

C175

SYNTHESIS OF ALLOSTERIC/ORTHOSTERIC HYBRID COMPOUNDS AS ANTAGONISTS FOR MUSCARINIC RECEPTORS

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Among the five subtypes of muscarinic receptors the amino acid sequence of the orthosteric acetylcholine-binding site is highly conserved so that drugs that target the orthosteric site generally lack therapeutically relevant subtype selectivity.[1] However, there is a second (allosteric) binding site that is less conserved among the different subtypes. Allosteric modulators that bind to this region have the ability to affect equilibrium binding of orthosteric agonists or antagonists.
Fusion of appropriate building block of ligand of both binding sites was shown to yield a new class of highly selective and active muscarinic receptor agonists that combines allosteric subtype selectivity with orthosteric receptor activation.[2] In order to translate this concept directly to muscarinic receptor antagonists, the orthosteric agonistic building block, i.e. iperoxo, was replaced by the orthosteric antagonists atropine and scopolamine, respectively.
Using [^{3}H]N-methylscopolamine ([^{3}H]NMS) as an orthosteric probe the binding affinities of the different compounds were determined at human receptors or mutant receptors, respectively, both being expressed in CHO cells. As the allosteric building blocks have highest affinity for the M_2 receptor subtype and lowest for M_5, binding affinity to these two subtypes was measured. The antagonist hybrid Naph6Atr displayed a relatively small but still significant M_2/M_5 subtype selectivity of 0.7 log units of affinity, whereas the antagonist atropine itself shows no appreciable selectivity. Additionally there is evidence that the appropriately designed antagonists also bind – in analogy to agonists – in a dualsteric mode at muscarinic receptors.
The proof-of-principle is provided by this study, the M_2/M_5 selectivity has to be optimized in the future. The synthesis of corresponding derivatives is in progress.

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C176

"TRIVALENT" QUINAZOLINIMINES: HIGHLY POTENT AND SELECTIVE BUTYRYLCHOLINESTERASE INHIBITORS

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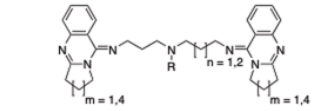
According to current research butyrylcholinesterase (BChE) compared to acetylcholinesterase (AChE) seems to represent an equally important target within the cholinergic system for treatment of cognition deficits in Alzheimer's disease (AD). Tricyclic quinazolinimines have previously been identified as a novel structural template for cholinesterase (ChE) inhibitors. Homobivalent quinazolinimines bridged by alkylene spacers proved to be nanomolar inhibitors of both AChE and BChE. The degree of BChE selectivity was dependent on alicycle ring size.

In this work, novel bivalent tricyclic quinazolinimines were synthesized: alicycle ring size, spacer length, and especially the chemical structure of the spacer (incorporating *N*-alkyl and *N*-acyl moieties) were systematically modified and investigated. Molecular modeling and docking procedures were applied to explain the data and probe the mid-gorge site of both ChEs.

Intensive and systematic investigation into the different structural parameters with special regard to the spacer structure identified a compound with eight-atom spacer, eight-membered ring alicycles and a 4-methylpentanoyl moiety as the acyl part as a highly potent (IC_{50} = 3 nM) and selective inhibitor of BChE (>250-fold selectivity over AChE). Docking demonstrated the importance of the spacer structure on the compounds' binding data characterizing them as "trivalent" inhibitors making use of the ChEs' mid-gorge binding site.



1: n = 1, m = 1, R = CH_3
2: n = 1, m = 1, R = $COCH_3$
3: n = 1, m = 4, R = CH_3
4: n = 1, m = 4, R = $COCH_3$
5: n = 2, m = 1, R = CH_3
6: n = 2, m = 1, R = $COCH_3$
7: n = 2, m = 4, R = CH_3
8: n = 2, m = 4, R = $COCH_3$
9: n = 2, m = 1, R = $COCH_2CH_3$
10: n = 2, m = 4, R = $COCH_2CH_3$
11: n = 2, m = 1, R = $COC(=CH_2)CH_3$
12: n = 2, m = 4, R = $COC(=CH_2)CH_3$
13: n = 2, m = 4, R = $COCH_2NHCOCH_3$
14: n = 2, m = 4, R = $COC(CH_3)_3$
15: n = 2, m = 4, R = $CO(CH_2)_3CH_3$
16: n = 2, m = 4, R = $COCH_2CH(CH_3)_2$
17: n = 2, m = 4, R = $COCH_2CH_2CH(CH_3)_2$

C177

2-AMINOTHIOPHEN-DERIVATIVES A NEW CLASS OF ANTAGONISTS OF THE GLUR6-RECEPTOR

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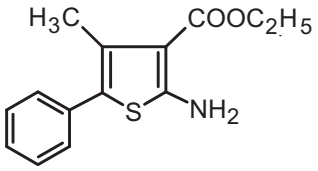
Kainate receptors are interesting potential targets for the development of new anti-epileptics. Among these receptors a special significance is ascribed to the kainate receptor subtype GluR6. This subtype is primarily expressed in the excitatoric pyramidal cells of the hippocampus. There are hints that the GluR6 and the GluR5 subtype play an opposing role in the hippocampal activation.
Compound **1** was our lead structure within the new class of thiophene derivatives with selective GluR6-antagonistic activity. Starting from **1** a number of compounds was synthesized and studied for their GluR5- und GluR6-inhibition in an in-vitro kainate-receptor assay. Thereby the structural division of the molecule into a hydrophobic (**2**: R3, R4) and a hydrophilic region (**2**: R1, NR2) was maintained. To get information about the role of the NR2-group for the receptor binding the electron density and the H-donor properties of this molecular position were varied. The activities of the new derivatives were compared with that of compound **1** (IC 50 = 0,75 µM, GluR6 >100 µM, GluR5 respectively).



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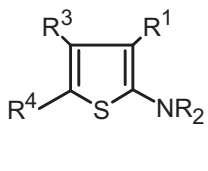

2

C178

N-ACETYL-5-ARYLALKOXYTRYPTAMINE ANALOGS: PROBING THE MELATONIN RECEPTORS TOWARDS MT_1-SELECTIVITY

Markl, Ch.[1], Attia, M. I.[1], Clafshenkel, B.[2], Julius, J.[2], Witt-Enderby, P. A.[2], Zlotos, D. P.[3]
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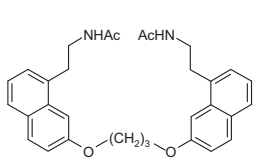
The neurohormone melatonin (MLT, R = Me) exerts its physiological actions mostly via activation of two G-protein-coupled MT_1 and MT_2 receptors. An accurate characterization of MLT receptor-mediated functions requires MT_1 and MT_2-selective ligands. While many series of MT_2-selective agents are known, pronounced MT_1-selectivity is still a challenge with only few examples reported up to date.[1] The common structural feature of MT_1-selective ligands is the presence of a bulky hydrophobic substituent in a position topologically equivalent to the MeO-group of MLT. In order to probe the MLT receptors for MT_1-selectivity, a series of MLT analogs obtained by the replacement of the ether methyl group with larger arylalkyl and aryloxyalkyl substituents was prepared. The most MT_1-selective agents were substituted with a $Ph(CH_2)_3$ or a $PhO(CH_2)_3$ group. Both compounds displayed higher MT_1-affinity and selectivity than the most MT_1-selective ligand **1** known up to date.



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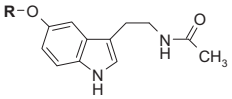

R = n-hexyl, $-(CH_2)_nPh$ (n = 1-5), allyl naphth-2yl, $-(CH_2)_2OPh$, $-(CH_2)_3OPh$, $-(CH_2)_3O$-(naphth-1yl), $-(CH_2)_3O$-(naphth-2yl), $-(CH_2)_3O$-(biphenyl), $-(CH_2)_3$-(phthalimide-2yl)

[1]Zlotos, D. P. *Arch. Pharm. Chem. Life Sci.* 2005*, 338.*

C179

DIRECT FORMATION OF FUSED 1,3-THIAZINE-2,4-DITHIONES: OBSERVATION OF A CARBON DISULFIDE MEDIATED THIONATION

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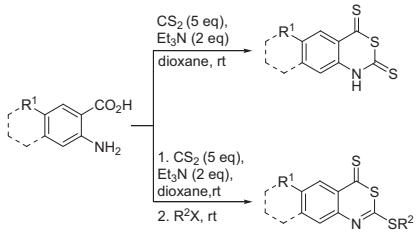

In the course of our investigations directed towards the synthesis of 3,1-benzothiazines[1] we explored a facile synthesis of 2*H*-3,1-benzothiazine-2,4(1*H*)-dithiones (trithioisatoic anhydrides)[2,3] or 2*H*-naphtho[2,3-*d*][1,3]thiazine-2,4(1*H*)-dithione solely from anthranilic acids or 3-amino-2-naphthoic acid and carbon disulfide, performed at room temperature in 1,4-dioxane in the presence of Et_3N. Corresponding 2-alkylsulfanyl derivatives were obtained in one-pot reactions under the same conditions after addition of alkyl halides. The convenient procedures to directly form trithioisatoic anhydride derivatives proceed in moderate to excellent yields with fast and easy work up.
The mechanism of the thiazine cyclization has been investigated with ^{13}C-labeled carbon disulfide to reveal that carbon disulfide was incorporated into the heterocycle and additionally acted as a thionation reagent. The fact that carbon disulfide can facilitate a carbonyl oxygen-sulfur exchange is an almost unique observation until today.[4]

[1] Ottersbach, P. A. et al. *Tetrahedron Lett.* **2010**, *51*, 2727. [2] Wagner, G.; Rothe, L. *Pharmazie* **1971**, *26*, 271. [3] For the reactivity of trithioisatoic anhydrides see, for example, [a] Leistner, S. et al., *Z. Chem.* **1972**, *12*, 289. [b] Leistner S, et al. *Monatsh. Chem.* **1983**, *114*, 915. [4a] Polshettiwar, V.; Kaushik, M. P. *J. Sulfur Chem.* **2006**, *27*, 353. [b] Brillon, D. *Sulfur Rep.* **1992**, *12*, 297.

C180

ENZYMATIC RESOLUTION OF AZO COMPOUNDS WITH QUATERNARY STEREOCENTERS

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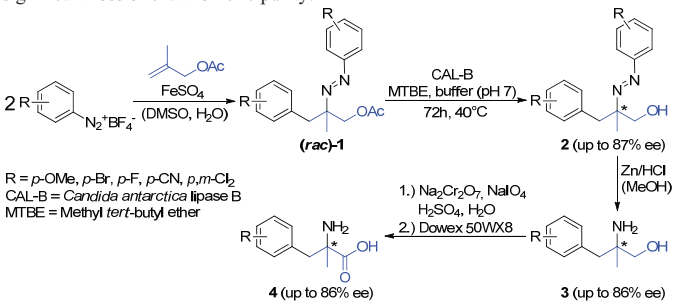
Only a few examples for the enzymatic resolution of compounds with quaternary stereocenters have so far been reported.[1] We have now developed a first two-step strategy to obtain enantiomerically enriched azo alcohols with quaternary carbon centers. Racemic azo compounds **1**, which are accessible by a flexible methodology developed in our group,[2] were further converted to alcohols **2** by enzymatic resolution with an enantiomeric excess up to 87%. Further conversion to amino alcohols **3** and to amino acids **4** has been shown to be possible without significant loss of enantiomeric purity.[3]



In this pioneering approach radical and enzymatic methods have been successfully combined to open up a new synthetic access to a wide range of pharmaceutically relevant substances.

[1] a) S. Acherar, G. Audran, N. Vanthuyne, H. Monti, *Tetrahedron: Asymmetry* **2003**, 2413–2418; b) L. F. Solares, R. Brieva, M. Quirós, I. Llorente, M. Bayodb, V. Gotor, *Tetrahedron: Asymmetry* **2004**, 341–345.
[2] a) M. R. Heinrich, O. Blank, S. Wölfel, *Org. Lett.* **2006**, *8*, 3323–3325; b) M. Heinrich, O. Blank, A. Wetzel, *J. Org. Chem.* **2007**, 476–484.
[3] A. Prechter, F. Dietz, H. Gröger, M. R. Heinrich, patent application **2010**.

C181

SYNTHESIS OF *N*- AND *O*-SULFONYLATED 5,6,7,8-TETRAHYDRO-BENZO[4,5]THIENO[2,3-*d*]-PYRIMIDINE/QUINAZOLINE DERIVATIVES AND OBSERVATION OF A N→O SULFONYL TRANSFER

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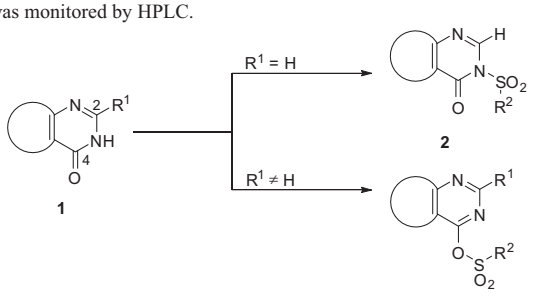
3-Phenylsulfonylquinazoline-2,4-diones were found as potent and covalently interacting inhibitors of chymotrypsin-type serine proteases.[1] The reactivity of their lactam carbonyl is increased by the electron-withdrawing arylsulfonyl group at the lactam nitrogen.[2] The direct conversion of the appropriate pyrimidine derivative with a sulfonyl chloride was considered to be the most convenient access to such compounds and was initially studied. Two possible products might be obtained in the course of this reaction due to the two nucleophilic sites (N-3 and O) of the pyrimidine ring. For the related alkylation of pyrimidine derivatives, an influence of the substituent in position 2 on the regioselectivity has been discussed.[3] We studied the regioselectivity of the direct sulfonylation of **1** and devised an alternative synthetic route to *N*-sulfonylated pyrimidinones **2**. Furthermore, a rearrangement was observed to produce **3** from **2**. The underlying kinetics was monitored by HPLC.



[1] Fukami et al. *Drug. Des. Discov.* **2000**, *17*, 69-84. [2] Maruoka et al. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3435-3439. [3] Hori et al. *Chem. Pharm. Bull.* **1990**, *38*, 1286-1291.

C182

NOVEL FLUOROETHYL-DERIVATIVES OF THEOPHYLLINE

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[1]Institut für Pharmazie, Universität Leipzig, Deutschland

The xanthine derivatives theophylline, 7-(2-hydroxyethyl)-theophylline (etofylline) and 7-(2-chloroethyl)-theophylline (benaphylline) are well explored antagonists of adenosine receptors as well as unspecific inhibitors of PDE-enzyms. Based on these mechanisms theophylline and etofylline are commonly used therapeutics for airway diseases like COPD and asthma.
We synthesized novel 7-fluoroethyl-derivatives of theophylline as electrosteres to etophylline respectively isosteres to benaphylline to complement the structure-activity-relationships at adenosine receptors. Starting from theophylline, we alkylated using a variety of 2-fluoroethylhalides whereby the yield was decreased by increasing the number of fluorine atoms in the alkyl chain. Depending on the base, 7-(1-fluoro-2-iodo-vinyl)-theophylline was isolated as side product using 2,2,2-trifluoro-1-iodethane undergoing an elimination of hydrogen fluoride. Cytotoxicity is investigated on human neuroblastoma-(SH-SY5Y), kidney-(HEK293) and hepatocyte-(HEPG2) cell cultures using the MTT cell vitality assay and a lactate dehydrogenase assay. Until now there are no cytotoxic effects also after long-term incubation (48h) in the highest concentration (100 µM) detectable. The inhibition of adenosine receptors is temporally tested using binding assays. Furthermore, we expect activities on PDE-isoenzymes.

C183

TRITYL RADICALS: SYNTHESIS AND ESR CHARACTERIZATION

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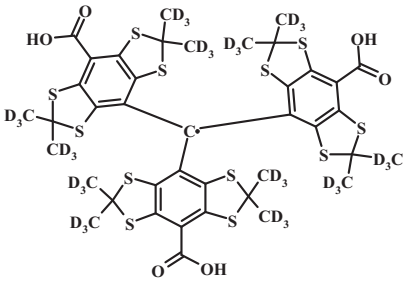
Electron Spin Resonance (ESR, EPR) has been proposed for many years as a suitable tool for oxygen and pH measurement. [1] However, current limitations include low sensitivity due to broad lines of the probes, the superposition of several radical species with overlapping lines, non reliable performance and rapid signal loss. [2] Suitably substituted triarylmethyl radicals (TAM) would be ideal [3], but their use has been severely restricted by the very difficult synthetic accessibility. Using the example of the deuterated TAM **1**, we present details of a synthetic procedure that addresses and solves major obstacles that were neglected hitherto [4], *e.g.* the preparation of the tetrathioarylether, the stability of the radical towards air, and factors influencing the shape and breadth of the ESR signal (solvent, conformation, concentration *etc.*).



References: 1. Lurie, D. A. & Mäder, K. (2005) Adv. Drug Deliv. Rev. 57:1171-1190. 2. Kempe, S., Metz, H. & Mäder, K. (2010) Eur. J. Pharm. Biopharm. 74:55-66. 3. Andersson, S. et al. (1996) US Patent 5530140. 4. Dhimitruka, I. et al. (2010) Bioorg. Med Chem. Lett. 20:3946-3949.

Poster
Klinische Pharmazie

K184

ASSOCIATION OF ANGIOTENSIN II TYPE 2 (AT2) RECEPTOR GENE POLYMORPHISM -1332G/A WITH LEFT VENTRICULAR DYSFUNCTION IN A COHORT OF PATIENTS PRESENTING WITH CARDIOVASCULAR SYMPTOMS

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[2] Clinical Pharmacology Unit, Institute of Experimental and Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Germany
[3] Department of Cardiology, University Heart Center, Hamburg, Germany

Background: The well-known biological functions of angiotensin II (Ang II), such as control of blood pressure, aldosterone secretion, vasoconstriction, and growth promotion, are elicited by activation of the ubiquitously expressed AT1 receptor. In contrast, the AT2 receptor is only expressed at low levels in various tissues of the adult organism. Nevertheless, it is upregulated during cardiac and vascular injury and seems to counterbalance the effect of the AT1 receptor in these pathological conditions. The polymorphism -1332G/A (rs1403543) located in a branch-point site of the AT2 receptor gene has been associated with altered receptor expression and left ventricular hypertrophy. **Objective:** We assessed the association of the -1332G/A AT2 receptor gene polymorphism with left ventricular function in a cohort of patients presenting with cardiovascular symptoms at the University Medical Center Hamburg-Eppendorf between 2003 and 2005. **Methods and Results:** In 608 patients, we determined the -1332G/A polymorphism of the AT2 receptor gene and analysed its association with left ventricular function. Because of the X-chromosomal location of the AT2 receptor gene, heterozygous females were excluded from the analysis. Moreover, we collected follow-up data on mortality and cardiovascular complications, such as myocardial infarction, stroke, and revascularization during a mean of three years after inclusion of the patients. In cross-sectional analysis, the prevalence for reduced left ventricular function was elevated in patients carrying the -1332A-allele [116 (47.2%) vs. 67 (34.9%); p=0.010]. In multivariate analysis adjusted for age, gender, BMI, hypertension, hypercholesterolemia, diabetes mellitus and nicotine abuse, the presence of the -1332A-allele was associated with reduced left ventricular function [OR 1.62 (95% CI 1.08-2.43; p=0.020)]. However, the gene variant was not associated with an increased incidence of mortality or cardiovascular complications in these patients.
Conclusion: The -1332G/A polymorphism of the AT2 receptor gene is associated with a higher prevalence of left ventricular dysfunction, but not with mortality or cardiovascular complications in a cohort of patients presenting with cardiovascular symptoms.

K185

INVESTIGATION OF ABSORPTION MODELS FOR NEVIRAPINE IN HEALTHY MALES TO SUPPORT MOTHER & NEWBORN DATA

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[2]Department of Pharmaceutical Biosciences, Uppsala University and [3]Division of Clinical Pharmacology, University of Cape Town
[4]Institute of Tropical Medicine, Charité-Universitaetsmedizin Berlin

Objectives: To reduce the risk of HIV transmission from mother to child nevirapine (NVP) was given as prophylaxis in Uganda. 62 HIV positive pregnant women and newborns received a single oral dose of NVP. Population pharmacokinetic (PK) analysis was performed to characterise NVP PK in mothers and newborns. Due to sparse data situation rich data of NVP in healthy male volunteers were used to describe complex absorption processes of NVP.
Methods: NVP-based prophylaxis consisted of 200 mg NVP tablets for women and healthy males and 2 mg/kg NVP syrup for newborns. 113 plasma samples of mothers and newborns as well as 95 breast milk samples were available for PK analysis of a combined model. 390 plasma samples of 26 volunteers were available to describe the PK of NVP in healthy males. Population PK analysis was performed using NONMEM™. Appropriateness of model fit and performance was guided by various diagnostic tools.
Results: Based on prior experiences for separate PK models [1] a combined population PK model for mothers and newborns was developed. First results suggest sufficient model performance. Due to the sparse data situation additional data of healthy males were used to describe complex absorption processes of NVP. A transit compartment model excelled zero-order and mixed-order absorption models. In a subsequent step, knowledge from population PK analysis of healthy males will be implemented in the combined PK model.
Conclusion: Despite of the sparse data situation a first combined PK model for mothers and newborns was developed. Additional data of healthy males will be used to characterise the complex PK of NVP more adequately, especially the absorption process. The final PK model could guide dosing regimes for newborns to assist prevention strategies for HIV transmission from mother-to-child.

References: [1] M. Frank et al. Population pharmacokinetic model development and evaluation after nevirapine administration to mothers and newborns. PAGE 18: 1484 (2009).

K186

DOXORUBICIN - STABILITY TESTING AND VALIDATION OF A HPLC-METHOD FOR A EUROPEAN PHASE II TRAIL IN CHILDREN

Kontny, N.[1], Krischke, M.[2], Lanvers-Kaminsky, C.[3], Schulze-Westhoff, P.[3], Boos, J.[3], Hempel, G.[1]
[1]Department of Pharmaceutical and Medical Chemistry – Clinical Pharmacy -, University of Muenster [2]Center for clinical trials (ZKS), University Hospital Muenster [3]Department of Paediatric Haematology and Oncology, University Children's Hospital Muenster, Germany

Background: Doxorubicin (Doxo) is a key component in the treatment of paediatric cancers. A better knowledge of the Doxo pharmacokinetics in children is needed because data on which to base current dosing regimens are limited. The EPOC consortium with participants from the UK, France, Germany and Italy conducts an investigator initiated trial (IIT) that aims to investigate the age-dependency in the clearance of Doxo in children. **Methods:** IITs often cannot set as high quality standards as drug approval studies. In our HPLC-method for the quantification of Doxo and Doxorubicinol (Doxol) in human plasma, however, all working steps including the validation are performed in compliance to good clinical laboratory practise (GLCP) and the EMA´s "Guideline on Validation of Bioanalytical Methods". Epirubicin is used as internal standard and analysis time is about 30 minutes. Applying a Purospher® STAR RP-18e column (Merck), a gradient elution with water and acetonitrile, both with 0.1% formic acid, is used. **Results:** To cover a concentration range from 5 to 1000 microgram per litre, we need only 100 microlitres of plasma to produce valid results. As required, the deviations between the mean accuracy values and the nominal values were less than 9% and less than 20% at the limit of determination. For precision the coefficients of variation did not deviate more than 11%. Dilution linearity, reproducibility and selectivity were demonstrated and we proved that there is no carry-over. Up to now, plasma samples and stock solutions are stable for 3 month; concentrations of stored samples did not decline more than 15% from concentrations of freshly prepared solutions. Future experiments are ongoing to investigate the long-term stability of Doxo and Doxol in plasma and stock solutions for one year. Furthermore, we plan to screen pre-analytical errors that can occur during sample collection. **Conclusion:** We managed to develop a GCLP-compliant method that requires only a small volume of blood. This allows us to get blood samples from very young children and, in addition to that, to draw blood by capillary blood sampling during Doxo infusion.

K187

LEUKOPENIA IN CANCER PATIENTS RECEIVING HIGH-DOSE CHEMOTHERAPY AND MYELOSUPPORTIVE TREATMENT

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[2]and Graduate Research Training program PharMetrX, Halle, Germany
[3]University of Bonn, Dept. Clinical Pharmacy, Bonn, Germany

OBJECTIVES: Myelosuppression is one of the most important dose-limiting adverse events in many anticancer regimens. During a clinical study 19 patients received a combination therapy of carboplatin, etoposide and thiotepa/ifosfamid including peripheral blood stem cell retransfusion (PBSCT) and G-CSF treatment. The objective of the current data analysis was to describe the leukopenic effect in this regimen using pharmacokinetic/pharmacodynamic (PK/PD) modelling.
METHODS: Individual pharmacokinetic profiles for the drugs were estimated and integrated into a PD model for myelosuppression [1] assuming an additive effect of the drugs on the proliferation rate of cells in bone-marrow (BM). Modelling and simulation activities were performed using NONMEM^{TM} VI, statistical analyses using R 2.10.
RESULTS: The median leukocyte count before therapy was $3.97x10^9$ cells/L (range: 1.75-14.75x10^9 cells/L). Median nadir counts of 0.08 x10^9 cells/L (range 0.02-0.14x10^9 cells/L) were reached after 236 h (±39 h), reflecting a grade 4 leukopenia. Recovery to a leukocyte count above $3x10^9$ cells/L (grade 1) was observed after a median time of 408 h (±75 h) for patients receiving PBSCT. PK parameter estimates for the drugs were in line with previous knowledge. The leukocyte time-course after nadir revealed a steep increase in concentration followed by a pronounced rebound after PBSCT. The mean maturation time of leukocytes in BM was 94.4 h for all patients except one, not receiving myelosupportive treatment.
CONCLUSIONS: It was possible to integrate the generated individual PK profiles of the drugs in the PD model for leukopenia. Estimation of the PD parameters was precise although visual predictive checks (VPCs) suggest an improvement of the model by integration of the myelosupportive therapy in order to understand and manage this dose-limiting toxicity better and improve future chemotherapy.
REFERENCES: [1] LE Friberg et al. Model of chemotherapy-induced myelosuppression with parameter consistency across drugs. J Clin Oncol 20:4713-21 (2002)

K188

LINEZOLID CONCENTRATIONS IN CYSTIC FIBROSIS PATIENTS: EVALUATION OF COMPETING PHARMACOKINETIC MODELS

Schaeftlein, A.[1,2], Keel, R.A.[3], Kuti, J.L.[3], Kloft, C.[1]
[1]Department of Clinical Pharmacy, Martin-Luther-Universitaet Halle-Wittenberg, [2] and Graduate Research Training program PharMetrX, Germany [3]Ctr. for Anti-Infective Res. and Dev., Hartford Hospital, Hartford , CT U.S.A.

Background: Linezolid, used for the treatment of serious infections, is thought to portray nonlinear pharmacokinetics (PK), whereby clearance decreases over time. This might be of clinical relevance for the special population of cystic fibrosis patients especially after multiple dosing. The objective of this analysis was to develop a PK model for this special population and compare the predictive performance with other suggested PK models.
Methods: A linear classical two compartment model, a nonlinear, Michaelis-Menten model [1], a linear/nonlinear elimination mixture model [2] and a model with time-dependent inhibition [3] of linezolid were compared for describing and predicting the PK of 8 cystic fibrosis patients after 600 mg bid p.o. and i.v., single and multiple dose administration, in a cross-over design. All data analyses were performed using the nonlinear mixed-effect modelling approach (NONMEM^{TM}). Model comparison was guided by the Akaike information criterion (AIC), goodness of fit (GOF) plots, and visual predictive checks (VPC).
Results: (1) The patient linezolid data were best described by the time-dependent inhibition model, based on the lowest AIC value (779) of all models (819-1080). (2) GOF plots showed that the Michaelis-Menten and the mixture model were less capable of describing the data compared to the other two models. (3) VPCs of the classical two compartment and time-dependent inhibition model suggested that the latter better predicted the time-course in this population.
Conclusion: The time-dependent inhibition model appeared to be most suitable to predict linezolid PK profiles of these cystic fibrosis patients.
References: [1]. S. Swoboda et al. Eur J Clin Pharmacol, 66:291 (2010) [2]. AK. Meagher, A. Forrest et al. Antimicrob Agents Chemother, 47:548 (2003) [3]. N. Plock et al. Drug Metab Dispos, 35:1816 (2007).

K189

DEVELOPMENT AND EVALUATION OF INSTRUMENTS FOR HOME-BASED MEDICATION REVIEWS

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Institutes of [1]Pharmacy, Clinical Pharmacy, [2]Psychology, and [3]Community Medicine, Ernst-Moritz-Arndt-University of Greifswald

OBJECTIVE: Patients with impaired mobility are a high-risk population for drug-related problems (DRPs) such as adverse side effects or drug-drug interactions. They are, therefore, in urgent need of specialized pharmaceutical care provided by their community pharmacy. Standardized instruments are necessary to successfully conduct home-based medication reviews. *METHOD:* First, a questionnaire for home-based pharmacist-conducted medication reviews and a guide for detection and classification of DRPs in a structured approach were developed. Both were evaluated by six experts in a Delphi-like consensus approach. The questionnaire was further discussed in patient focus groups, while reliability of the guide was assessed by pharmacy students in their last year of education. *RESULTS:* By involving experts and patient focus groups standardized versions of questionnaire and guide were aimed for. In the first of a two-rounded Delphi-like consensus approach experts agreed at an average to 104.2, generally agreed to 8.5 and did not agree to 6.3 out of 119 aspects of a scoring list for the questionnaire. Concerning the guide, 167.6 out of 177 aspects were accepted, 2.7 generally accepted and 6.7 not accepted. Reliability analysis revealed that in a constructed patient case carrying ten DRPs in a first round including 17 students, 3.3 +/-1.4 (range 1-6) DRPs were identified. In a second round with an improved version including 32 students, 4.0 +/-1.5 (1-7) DRPs were detected. Interrater reliability was assessed among four groups of 5 students (3.6; 5.2; 3.2; 3.6 mean detected DRPs), and increasing group sizes of 2 (3.0) 5 (3.4), and 10 (4.0) students. In addition, a real patient case carrying two DRPs was assessed. Of all 35 students 28 (80%) detected both DRPs. Detection of both DRPs was also accomplished by 4/5 in 3 groups and 3/5 in 1 group. Group size did not affect rating efficiency as 2/2, 4/5, and 9/10 students identified both DRPs within the real patient. The Inter correlation coefficient (ICC) is 0.67. *CONCLUSION:* Both instruments are reliable to assess DRPs in home-based medication reviews of patients with impaired mobility and can be implemented in prevalent models of pharmaceutical supply which are required to be established.

K190

RANDOMIZED TRIAL COMPARING COMPLIANCE-MONITORING WITH ELECTRONIC OTCM-BLISTER PACKAGES AND MEMS®

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Background: The OtCM-system (Objective Therapy Compliance Measurement) is an innovative method for electronic compliance monitoring based on standard blister packages. So far MEMS® (Medication Event Monitoring System, Aardex, Switzerland), which works with conventional medicine bottles equipped with an electronic cap, represents the state-of-the-art technology for objective compliance measurement. Whenever the MEMS® cap is opened or a dosage form is taken from an OtCM-activated blister package, date and time are electronically documented. However, the quality of information gained is different. Each date/time combination retrieved from the OtCM-system correlates with removal of a single dose. In contrast the MEMS® cap openings can go along with removal of several doses at once or no dose. To verify the amount of actually removed doses the remaining medication is to be counted (pill count). The aim of this open, randomized, two-armed, prospective study was to compare both systems regarding quality of data, expenditure of time, functionality and practicability and to evaluate suitability and validity of the OtCM-system in clinical practice.
Material and methods: The compliance measurement methods were compared in patients with potassium chloride replacement therapy over a four-week observation period. In parallel group design patients received Rekawan® retard capsules either in OtCM-activated blister packages or in MEMS® containers. At the end of the observation period patients were asked to fill out a questionnaire concerning the practicability of the respective system. Expenditure of time for preparation of the study medication and data analysis was documented.
Results: 34 patients participated in the study over an average of 22 days. Taking- and dosing-compliance rates were similar in both groups. However, in the MEMS-group the electronic data had to be substantiated by pill count and to be adapted when indicated. Functionality and practicability of the OtCM system were proven. Expenditure of time for preparation of medication and evaluation of data is less for the OtCM-system.
Conclusion: OtCM-activated blister packages provide more reliable registration of medication removals and more valid information about dosing-compliance than the MEMS® caps.

K191

POTENTIALLY INAPPROPRIATE MEDICATIONS – A GERMAN-IRISH COMPARISON IN THE RESIDENTIAL HOME CARE SETTING

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Introduction: The prescribing of potentially inappropriate medications (PIMs) is a common problem. To determine the quality of inappropriate prescribing in the elderly (≥ 65 years) living in a residential home setting, a comparative study between two European countries (Germany and Ireland) using the STOPP screening tool was conducted. **Methods:** Prescriptions, diagnoses and biochemical data of residents from 4 nursing homes in the South of Ireland (n=127) were recorded. Corresponding to age and gender these data were matched with data from 7 nursing homes in Münster (n=370). In total 115 matched pairs of residents were reviewed in using the STOPP screening tool to assess the prevalence of PIMs. **Results:** Each cohort consisted of 102 (88.7%) women and 13 men (11.3%), mean age 83.8 ± 7.4 years. A total of 1308 prescriptions were recorded in the Irish cohort (mean 11.8 ± 3.8), which corresponded to 1422 documented diagnoses (mean 11.4 ±4.0). The German cohort received a total of 1419 prescriptions (mean 12.2 ± 4.9), corresponding to 1232 diagnoses (mean 10.6 ± 4.3). The total number of PIMs detected in the Irish group was 267 (median 2, IQR 1-4). 87 Irish residents (75.7%) received at least one PIM. In the German cohort the total number of detected PIMs was 170 (median 1, IQR 0-2), while 76 residents (66.1 %) received at least one PIM. The amount of the criterion B7 (long-term use of long-acting benzodiazepines) was 14 (5.2%) in the Irish cohort. In the German cohort there was only 1 patient (0.6%), receiving a long-acting benzodiazepine, but in the German cohort the usage of Antipsychotics was higher. **Conclusions:** In both countries the prevalence of PIMs is high. A lower number of PIMs were identified in the German residential home setting. This might be due to a different prescribing practice in the two countries. The Irish cohort has a higher use of long-acting benzodiazepines, counted as PIMs. Antipsychotics, which were mostly not identified as PIMs, are more common in the German cohort. Further research has to be done to evaluate the reasons of these outcomes.

K192

PAIN IN NURSING HOME RESIDENTS – CHALLENGES AND OPPORTUNITIES FOR APPROPRIATE THERAPY

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Background
Pain is one of the most important syndromes in the elderly population, particularly in nursing home residents (NHR). Our aim was to evaluate pharmacotherapy of pain in nursing home residents in Berlin and Brandenburg, Germany.

Methods
We conducted a study to interview NHR and to assess pain and pain medication. Quality of pain medication was assessed with the "Pain medication appropriateness scale" (PMAS). In this scale, developed in 2006 in the US, pain control is classified on a percentage scale in which a score < 67% indicates poor pain control. Data on pain intensity, all medication prescribed to the subjects, and surgery procedures or injuries during the last 3 weeks were collected.

Results
Overall, 321 residents having pain or receiving scheduled pain medication were eligible for analysis. From these, 196 were able to report pain themselves during the interview, 98 NHR with cognitive impairment showed pain-associated behavior, and 27 individuals received scheduled pain medication but did not complain about pain.
Scheduled analgesics were prescribed to 179 residents; the most frequently prescribed drugs were metamizol, fentanyl and tramadol.
For the 321 residents the mean PMAS score was 49%. Overall, about 75% of all subjects had a PMAS score below 67%. Furthermore, two thirds of NHR with prescribed opioids according to WHO step 3 were not treated with laxatives for prophylaxis of constipation.

Conclusion

Our results demonstrate some problems in pain pharmacotherapy in NHR in Germany. These results should serve as a basis for practice guidelines aiming at eliminating these deficits to improve pain therapy in the elderly.

K193

MULTIDISCIPLINARY INTERVENTION TO OPTIMISE TUMOR PAIN THERAPY AND SYSTEMATIC MONITORING OF ADVERSE EVENTS

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Introduction
A high fraction of tumor patients are not treated appropriately. Clinical pharmacists and computerised clinical decision support systems (CDSS) enhanced therapeutic outcomes in many areas. We aimed to assess (i) the impact of a multidisciplinary intervention in tumor pain therapy based on a newly developed CDSS and (ii) the prevalence of analgesia-related adverse events (AEs) to identify additional fields that should be addressed by further interventions**.**
Patients and Methods
After ethical approval a two-step study was performed in consecutive tumor patients: (i) a prospective controlled study in two groups (control, intervention) to decrease guideline deviations in pain therapy (primary endpoint) by a pain management based on a CDSS, (ii) an observational study to identify analgesia-related AEs by a daily monitoring procedure. Fisher's-Exact-Test and Mann-Whitney-U-Test were used as appropriate (significant with $p<0.05$).
Results
(i) Out of 279 recommendations, 85% were fully accepted by the physicians. The number of patients with guideline deviations decreased from 37 to 7 ($p<0.001$, n=50 consecutive patients per group) and of those treated with co-analgesics increased from 23 to 33 ($p=0.04$). In the intervention group, median pain assessed by visual analogue scale decreased at rest from 3 (Q25%-Q75%: 2-5) on admission to 1.5 (1-2) at discharge ($p<0.01$) and during physical activity from 7 (5-9) to 2.5 (1-5; $p<0.001$). (ii) In 106 consecutive patients 11% suffered from vomiting, 41% from nausea, and 36% from constipation. During their hospital stay 31% and 65% of all pain patients were not treated with an antiemetic or a laxative, respectively.
Conclusion
A multidisciplinary intervention based on a newly developed CDSS ameliorates pain treatment. A high fraction of analgesia-related AEs and a high rate of patients not appropriately treated with antiemetics and laxatives indicated that supportive therapy should be addressed in further interventions (e.g. by an optimised CDSS).

K194

MEDICATION ERRORS IN PEDIATRIC INPATIENTS: IDENTIFICATION AND PREVENTION BY A CLINICAL PHARMACIST

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Background Pharmacotherapy in children implies complex and individual treatment regimens. Processes of drug prescription or administration drugs in children give rise to medication errors. In order to increase safety in pediatric pharmacotherapy, susceptible domains for these medication errors have to be identified and ideally prevented to avoid harm. **Methods** During a 12-month period a clinical pharmacist was assigned to review medication order sheets twice a week and participated in medical rounds once a week on the pediatric ward for cardiology and pneumology at the university hospital of Duesseldorf. Due to ethical reasons, the medication errors were immediately discussed by the clinical pharmacist and the responsible physicians to avoid harm to the patients. Then, an intervention was made and the prescription was corrected. Medication errors were collected, number and types were determined, classified with a modified PCNE-System1 and the severity of the medication errors according to the NCC MERP index2. **Results** The clinical pharmacist reviewed 413 patient charts with 2322 prescriptions and 214 different drugs. Mean age of the patients was 6.5 years. Out of the 2322 prescriptions 393 medication errors were identified. The types of error classified with the PCNE-System1 were: "documentation" (363) and "therapy" (30). According to the severity, the errors could be classified as "potential errors" (44%), "errors which did not reach the patients" (48%), "errors reached patients without harm" (8%) and "errors need monitoring" (1%). **Conclusion** There are profound problems in the quality management of routine drug prescription in the hospital. Our analysis revealed that one out of six prescriptions needed to be corrected. According to the clinical pharmacist´s intervention, 92% of errors could be avoided.

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[2] National Coordinating Council for Medication Error Reporting and Prevention. Index for Categorizing Medication Errors. http://www.nccmerp.org/medErrorCatIndex.html (accessed 30/06/2010).

K195

MRP INHIBTORS INCREASE PLATINUM ACCUMULATION UPON EXPOSURE OF TUMOR CELLS TO OXALIPLATIN

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The mechanisms leading to oxaliplatin resistance resemble those of cisplatin resistance, including lower intracellular platinum accumulation, increased intracellular glutathione (GSH) levels and efflux via multidrug resistance-associated proteins (MRP). To further elucidate the efflux mechanism of platinum complexes via MRP1 and MRP2, we investigated the effect of MRP inhibitors on platinum accumulation and determined intracellular GSH levels under various conditions.
Cells of the human ileocecal colorectal adenocarcinoma cell line HCT8 and its oxaliplatin-resistant variant HCT8ox were incubated with oxaliplatin (100 µM) and the GSH-depleting agent buthionine sulfoximine (BSO) as well as different concentrations of the MRP inhibitors Gü83[1] or indometacin. Intracellular platinum concentrations were determined using graphite furnace AAS. GSH was quantified after derivatisation with 2,3-Naphthalenedicarboxaldehyde using a microplate assay. Platinum and GSH concentration were related to the protein content determined by the bicinchoninic acid (BCA) assay. The effect of BSO on oxaliplatin cytotoxicity was assessed using the MTT assay.
Coincubation of HCT8 and HCT8ox cells with oxaliplatin and indometacin or Gü83 led to an increased accumulation of platinum. The effect was shown to be concentration-dependent. The addition of oxaliplatin and/or Gü83, however, had no effect on the intracellular GSH content. Depletion of intracellular GSH levels by addition of BSO prior to oxaliplatin addition led only to a slight increase in oxaliplatin cytotoxicity and to no change in platinum accumulation.
The observed increase of platinum accumulation indicates that MRP1 and/or MRP2 contribute to platinum efflux from the cells investigated and is not limited to resistant cells. The role of GSH still remains unclear. Further investigations are required to identify the chemical species transported.
[1]Leyers S et al. Bioorg Med Chem Lett 2008; 18: 4761–3

K196

CONTRIBUTION OF Na^+/K^+-ATPASE TO PLATINUM ACCUMULATION IN OVARIAN CARCINOMA CELLS

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Cisplatin is one of the most effective drugs in the treatment of ovarian cancer. However, its therapeutic potential is limited by intrinsic or acquired resistance. A frequently observed mechanism of resistance is decreased cellular accumulation of cisplatin. In addition to passive diffusion, platinum uptake is also mediated by active or facilitated transport. One of the transporters assumed to be involved in the intracellular accumulation of cisplatin is Na^+/K^+-ATPase, which utilizes ATP hydrolysis as an energy source.
In order to investigate the role of Na^+/K^+-ATPase in cisplatin uptake in the ovarian carcinoma cell line A2780 and its cisplatin-resistant variant A2780cis, we assessed the influence of ouabain, a specific Na^+/K^+-ATPase inhibitor, and oligomycin, a depletor of intracellular ATP levels, on platinum accumulation.
Intracellular platinum concentrations (ng Pt/µg protein) were measured by flameless atomic absorption spectrometry (FAAS). The respective protein concentrations were measured with the bicinchoninic acid (BCA) protein assay.
Pretreatment with different concentrations of ouabain for 1 hour led to a decreased platinum accumulation in the sensitive A2780 cell line up to 33.7 ± 8.8 % (mean ± SEM; n = 4). However, no influence of ouabain on platinum uptake was observed in the resistant A2780cis cells.
Additionally, we examined the accumulation of cisplatin in oligomycin-treated cells. Under the conditions of ATP deficiency, the intracellular platinum accumulation after 60 minutes of incubation was significantly reduced to 53.9 ± 10.4 % and 72.7 ± 10.8 % (mean ± SEM; n = 5) in A2780 and A2780cis cells, respectively, compared to standard culture conditions.
Our results indicate that cisplatin is to some extent taken up by energy-dependent processes in the sensitive A2780 cells, whereas cisplatin uptake in the resistant A2780cis cells seems to be less dependent on intracellular energy sources. In addition, Na^+/K^+-ATPase may be directly or indirectly involved in cisplatin uptake in A2780 cells in contrast to resistant cells. Interestingly, the ATP-depletor oligomycin reduced the accumulation of cisplatin to a higher extent compared to ouabain, indicating the existence of other energy-dependent processes beside Na^+/K^+-ATPase regulating cisplatin uptake.

K197

PHARMACOKINETICS AND PHARMACODYNAMICS OF SUNITINIB AND SU12662 IN COLORECTAL CANCER PATIENTS

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Background. Sunitinib has been shown to influence the plasma concentrations of various circulating proteins which can serve as biomarkers for its anti-angiogenic properties. In this study plasma concentrations of sunitinib, its active metabolite (SU12662) and three biomarkers were determined in patients with metastatic colorectal cancer patients within the clinical study C-II-005. A thorough understanding of the dose-concentration-effect relationship of sunitinib is a prerequisite for the development of a biomarker-guided treatment optimization strategy.
Methods. Patients received 37.5 mg sunitinib daily in addition to FOLFIRI (n=17). Plasma concentrations of sunitinib and SU12662 were determined by LC-MS/MS and those of VEGF-A, sVEGFR-2, and sVEGFR-3 by immunoassays. Plasma concentrations and biomarker response were compared with a dataset previously obtained in 12 healthy volunteers (Lindauer et al. 2010).
Results. After one day 'on treatment' no differences in the plasma concentrations of the active drug relative to the given dose were observed for both populations. Plasma concentrations of VEGF-A increased immediately after administration exhibiting a large inter-individual variability. Maximum response of the soluble receptor levels was observed at the end of each cycle with concentrations of 52-94% (sVEGFR-2) and 32-96% (sVEFGR-3) of the respective baseline values. All biomarker levels returned to baseline after two weeks 'off treatment'.
Conclusion. Plasma concentrations of sunitinib and SU12662 as well as the observed changes in biomarker levels seemed to be comparable between the populations in relation to the duration of sunitinib treatment. The PK/PD models previously developed for healthy volunteers are currently adapted to describe concentration-effect relationships also in cancer patients.
Reference. Lindauer A et al. Pharmacokinetic/pharmacodynamic modeling of biomarker response to sunitinib in healthy volunteers. Clin Pharmacol Ther 2010; 87:601-8

K198

HOMOCYSTEINE AS BIOMARKER IN A PK/PD MODEL OF METHOTREXATE IN YOUNG ALL PATIENTS

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Objectives: Elevated homocysteine concentrations have been associated with neurotoxic symptoms upon chemotherapy with methotrexate (MTX). The aim of this study was to develop a PK/PD model based on plasma MTX and homocysteine concentrations measured in patients with acute lymphoblastic leukemia (ALL) as a basis for the development of improved dosing regimens with a lower risk of neurotoxicity.
Methods: Based on methotrexate and homocysteine plasma concentration data from 388 ALL patients of the TOTAL XV study [1] a PK-PD model was built with NONMEM 7.1 using the FOCE interaction method. Several compartmental and indirect response models were investigated to describe the PK/PD relationship. Body size, age, sex and renal function were investigated as potential covariates on the model.
Results: The PK of MTX could be described by a two-compartmental model, parameterized by CL, V1, Q and V2. Considering the wide range of age (1-18 years) and the heterogeneous degree of maturation in the population an allometric scaling was included. Creatinine clearance was positively related to MTX CL ($p < 0.001$). The relationship between MTX and homocysteine concentrations could be described by fitting an indirect response model with impaired elimination of homocysteine. A lower elimination rate constant for homocysteine (kout) was positively associated with MTX concentrations ($p < 0.001$) using an inhibitory Emax model.
Conclusions: Our semi-mechanistic PK-PD model describes the methotrexate and homocysteine concentrations of young ALL patients. The model is currently evaluated.
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K199

IS THE INTAKE OF STANDARDISED GINKGO BILOBA EXTRACT ASSOCIATED WITH A BLEEDING RISK?

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Background: During Ginkgo biloba leaf extracts intake, there have been several case reports of unexpected bleeding. Despite several studies of very different quality and to provide a higher level of evidenence, a systematic review and meta-analyses were undertaken to determine the effect of standardised Ginkgo biloba extract (GBE) on outcomes of haemostasis associated with risk of bleeding.
Methods: The Medline, Embase, SciSearch and Cochrane CENTRAL databases were searched to identify all randomised controlled clinical trials measuring parameters of primary or secondary haemostasis. Inclusion criteria were p.o. standardised GBE medication for at least one week. For statistical analysis, the software RevMan® 5 (The Nordic Cochrane Centre, Copenhagen) version 2008 was used. According to the type of continuous data three statistical methods were used: weighted mean difference (WMD), standardised mean difference (SMD) and generic inverse variance (GIV) [1].
Results: A total of 1979 adults (87% diseased patients), receiving GBE or placebo was investigated. Random-effects models of effects on baseline change or mean difference [95% CI] showed a positive effect of GBE on blood flow (reduced blood viscosity: WMD -1.03 mPa sec [-1.29;-0.78]), but no evidence of any effect on thrombocyte aggregation and coagulation (ADP-induced thrombocyte aggregation: WMD -0.35 % [-15.16;+14.46], fibrinogen: GIV -1.32 mg/dL [-10.66;+8.01], PT: SMD 0.00 [-0.09;+0.09], PTT: WMD -0.42 sec [-0.92;+0.09]). Subgroup analyses revealed a statistically significant reduction in PTT for subgroups receiving high-dose GBE of ≥240 mg/d (WMD -0.45 sec [-0.83;-0.08] and for studies only including patients vs. healthy volunteers (WMD -0.61 sec [-0.95;-0.27], respectively, both without being of clinical relevance.
Conclusion: Based on these meta-analyses of outcomes of haemostasis, comparison between GBE treatment and placebo groups did not indicate a higher risk of bleeding associated with standardised Ginkgo biloba leaf extracts, ultimately contributing to an informed evaluation of (self-)medication for patients.

References: [1] Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Intervention. The Cochrane Collaboration 2008; Version 5.0.1. Available from: URL: http://www.cochrane-handbook.org/, accessed Feb. 4, 2010.

K200

DEVELOPMENT OF A MULTIPROFESSIONAL TUMOR THERAPY MANAGEMENT

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Introduction: Systemic cancer therapy is complex and associated with a high incidence of adverse drug events. The aim of the project is to define the role of physicians, pharmacists and nurses in a multiprofessional tumor therapy management model.
Methods: Focus group meetings were held to identify different tasks as part of the multiprofessional tumor therapy management model. The modified Delphi technique was used to allocate these tasks to physicians, nurses and pharmacists by local cancer care teams. Physicians, nurses and pharmacists have been approached nationwide by the 'Deutsche Krebsgesellschaft', Germany's largest multiprofessional association in oncology, via an online questionnaire to assess the acceptance of the presented multiprofessional tumor therapy management model. Additionally the professions' perceptions on benefits and problems of multiprofessional teamwork were explored.
Results: Two focus group meetings of six clinical pharmacists and one moderator identified 38 multiprofessional tumor therapy management tasks. Two Delphi rounds with 12 participants (five nurses, four physicians, three pharmacists working together in three different cancer care teams) allocated these tasks to the different professions. The results of the online questionnaire show that the proposal was found to be reasonable (79% agreement, 11% disagreement; n=344), practical (65% agreement, 15% disagreement; n=337), time-saving (60% agreement, 15% disagreement; n=333), quality-enhancing (67% agreement, 15% disagreement; n=338) and feasible (68% agreement, 10% disagreement; n=335).
Conclusions: Our multiprofessional tumor therapy management model was generally accepted by the different professions. Moreover, problems and benefits of teamwork stated by the participants emphasized the need to (re)structure multiprofessional teamwork. Based on our model, more specialized models concerning e. g. medication adherence and supportive therapy strategies such as fatigue and nutrition can be developed and implemented according to the patient's needs.

K201

ADVERSE DRUG EVENTS IN GERMAN NURSING HOMES

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Increasing age is frequently linked with multiple chronic diseases and polymedication. Furthermore, aging-related physiological changes may affect the pharmacokinetics and pharmacodynamics of administered drugs increasing the risk of adverse drug reactions. International studies show that drug-related problems are common in elderly care facilities leading to medical and economic consequences. However, no systematic study has been conducted in German nursing homes so far.

In this prospectively designed cross-section analysis 2 clinical pharmacists surveyed the medical documentation of 789 nursing home residents in North Rhine Westphalia, Germany. Potential drug-related incidents were evaluated in cooperation with experts in clinical pharmacology and geriatrics to identify the presence, severity, and preventability of adverse drug events as well as the effects on the resident. This analysis is the first part of the study entitled "Drug Therapy Safety in Nursing Homes – cross-section analysis and feasibility of a multidisciplinary approach" which is funded by the Federal Ministry of Health (BMG) as part of the national campaign on "Medication safety (AMTS)".

In total, 102 adverse drug events were detected over a 30 day period of which 62.7% (64) newly occurred during that period resulting in an overall rate of 8.11 adverse drug events per 100 resident-months. 57.8% (37) out of the 64 events were judged preventable. Of all adverse events 30% were classified according to CTCAE as severe, disabling or life-threatening leading to a hospitalization rate of 27.3%. Adverse drug reactions most commonly happened upon neurological/ psychiatric (36%) and cardiovascular (27%) medications as well as antiinfectives (12%). Errors associated with adverse drug events occurred mainly at the stages of interdisciplinary communication, drug administration and monitoring in association with necessary dose adjustments.

K202

A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR HIGHDOSE LONGTERM INFUSION CARBOPLATIN

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Objectives: Carboplatin (CARBO) is a widely-used potent cytostatic drug for different malignancies in adults and children. Carboplatin is a lipophilic substance (-1,5 log Units) with a molecular weight of 373,26g/mol and a unbound-fraction of about 80% in Plasma. The main excretion route is via glomerular filtration. It' s pharmacokinetic is well-characterized with a high interpatient variability depending on the individual renal clearance. Cytostatic action and inverse action (mainly thrombocytopenia) are strongly associated with the achieved Carboplatin-Area under the curve (AUC). The aim of the current project was to evaluate a generic PBPK model for high-dose carboplatin to predict the individual systemic drug exposure.

Methods: The simulations of carboplatin were performed by using the software PK SIM® and MoBi®(both BAYER TECHNOLOGY SERVICES, Leverkusen). The model was developed and evaluated by using concentration-time profiles from patients receiving highdose carboplatin (Eickhoff PhD Thesis 2000). The collective consists of 7 patients with ovarian cancer, with an age of 24 to 59 years. The aim AUC during this Carboplatin high-dose therapy was 8,2-12 (mg/ml*min) wich should be reached by a dosage of 361-985mg/m² given as a 24h long-term Infusion. The model was evaluated with data sets of a group of 4 individuals (age 10,3-25,8 years) with different cancers (Eickhoff PhD Thesis 2000). They received doses from 1200mg- 4000mg Carboplatin (AUC 5,6-12 mg/ml*min) over a 96h long-term infusion.

Results: The integration of an accumulation of the drug in the red blood cells (Mandal et al. 2004) and the individal adjustment of the clearance, optimized with the options of the MoBi® Software, lead to a good correlation of the predicted with the observed data. The predictions of the pharmacokinetics in different age groups by the PBPK model were in good agreement with observed data. The concentration-time-curves (plasma and unbound CARBO) visualize the time-dependent changes in the plasma-protein binding of the platinum species.

K203

A VALID METHOD FOR QUANTIFICATION OF CYTOKINES FROM MICRODIALYSATE CONTRIBUTING TO BIOMARKER PROFILING

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Background: Cytokines (Mr 8-80 kDa) as immunomodulatory proteins are secreted by immune and tissue cells (e.g. fibroblasts) mediating all types of immune response such as inflammation, infection and allergic reactions. However, cytokine concentrations in plasma in most cases do not represent concentrations in irritated tissues. Microdialysis (MD) as a minimal invasive technique enables the determination of analytes in the interstitial fluid (ISF), i.e. at the site of inflammation. For quantification of cytokines in small sample volumes of microdialysate a reliable, valid bioanalytical assay was to be developed.
Methods: Microdialysate was sampled from 4 healthy volunteers using CMA 66 Linear MD Catheters (100 kDa cut-off), CMA 107 MD Pumps, CMA 106 Syringes and as MD perfusate Ringer's solution (RS) or a mixture of RL and human albumin solution (HA) (ratio 9+1). These solutions were also investigated as proxy matrices for microdialysate due to its very limited availability. All matrices were spiked with same amounts of the four model cytokines interleukin 6, 8 and 10 (IL-6, IL-8, IL-10) and tumour necrosis factor alpha (TNF-α) and measured using BD™ Cytometric Bead Array (CBA) and BD FACSArray™ Bioanalyzer.
Results: A robust, rapid flow cytometry (FCM) based multiplex assay was developed, allowing the simultaneous determination of IL-6, -8, -10 and TNF-α from sample volumes of 25 µL in the concentration range of 6-6000 pg/mL (for higher concentrations dilution integrity was demonstrated). RL+HA (9+1) emerged as a suitable proxy matrix for microdialysate. Validation was performed meeting international requirements[1, 2] (accuracy (%RE) and precision (%CV) ≤20%) and further in-house criteria.
Conclusion: The developed and validated method allows quantifying cytokines from microdialysate as a first step to facilitate MD investigations in healthy volunteers and patients. As future perspective, MD feasibility for the monitoring of cytokines as biomarkers for immune responses and therapy outcomes should next be demonstrated in pre- and clinical investigations.
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K204

REPAIR OF TOPOISOMERASE II ALPHA INDUCED DOUBLE STRAND BREAKS IS CELL-TYPE AND DRUG DEPENDENT

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BACKGROUND: The induction of DNA double strand breaks (DSB) is the active principle of topoisomerase II alpha inhibitors that are commonly used in chemotherapy. Cells actively respond to such severe damage by triggering cell cycle checkpoints, DNA damage repair and bypass pathways and eventually the cell death machinery. Thus, the outcome of therapy depends on the nature of the DNA lesions and on cell-type specific abilities to initiate and complete these processes. Also, these processes contribute to interpatient variability of chemotherapeutic response. The ultimate goal of our investigations is therefore to determine if elements of the cellular DNA damage response qualify as markers to guide individualised cancer therapy. To this end, we asked whether and how DNA double strand break repair affects the sensitivity of three tumour cell lines of different origins towards topoisomerase II alpha inhibitors with different mechanism of actions, doxorubicin and etoposide.
METHODS: The human intestinal cell lines CACO-2 and HT29 as well as the human acute monocytic leukaemia cell line THP-1 were exposed to etoposide and doxorubicin and subsequently maintained for defined periods of time in drug-free medium to allow DNA damage response. We assessed the time course of DNA damage (comet assay), clonogenicity (colony formation) as well as expression of proteins relevant to DNA repair (western blot and gene expression analysis).
RESULTS: Although both drugs caused the same initial level of DSB in THP-1 and CACO-2 cells, doxorubicin-induced DNA damage persisted whereas etoposide-induced strand breaks were rapidly repaired. Clonogenicity of CACO-2 cells after treatment with doxorubicin or etoposide (0.5-50 µM/L) for 3 h correlated with their ability to repair DSB, which is in contrast to our results with HT29 cells where this correlation is missing. DNA damage response involved differential expression of proteins relevant to DNA repair.
CONCLUSIONS: We assume that mechanisms and efficiency of DSB repair are dependent on the cell type and the DSB-inducing drug, because a direct correlation between DSB and cell death is present in some, but not in all cell type/drug combinations. The present, as well as previous results suggest that DSB induced by non-intercalating versus intercalating topoisomerase II alpha poisons are repaired by different mechanisms.

Poster
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P205

TRADITIONAL CHINESE MEDICINAL DRUGS INHIBITING BETA-AMYLOID AGGREGATION IN *C. ELEGANS*

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Alzheimer's disease (AD) is a neurodegenerative disorder and the most common form of dementia. It mostly occurs in elderly people, which is a part of human population that is growing due to the improved living conditions and medical care. Therefore also the number of AD patients is growing. Although AD is an increasing social and health problem, there is no therapy available to cure this disease.
Toxic beta-amyloid ($A\beta_{42}$) is one of the key proteins in the pathogenesis of AD. It is a cleavage product of amyloid precursor protein (APP). Aggregates of $A\beta_{42}$ build the core of the senile plaques, which are one of the morphological features found in the brains of AD patients. According to the amyloid cascade hypothesis, the accumulation and aggregation of Aβ are the first events that lead to AD symptomatic.
The aim of this study is to find new substances that could inhibit the aggregation of $A\beta_{42}$ and hence could be used to develop new drugs against AD. Drugs from traditional Chinese medicine (TCM) were chosen as a possible source for such substances. In TCM phytotherapy has an important role. Secondary metabolites of plants have many applications in the medicine due to their biological activities. The natural product galantamine, which inhibits the enzyme acetylcholine esterase, is already used in symptomatic therapy of AD.
A transgenic strain of *C. elegans* – CL2006 – was used as a model organism. This strain expresses the human $A\beta_{42}$ peptide in its muscles, where it forms $A\beta_{42}$ deposits similar to the senile plaques found in AD patients. Therefore it is a convenient model for studying the aggregation of $A\beta_{42}$ and molecular interactions associated with this process. The worms were treated with plant extracts and the effect on $A\beta_{42}$ deposits was assessed by microscopic evaluation.
Several plant extracts decreased the number of plaques observed. These extracts were also studied for their antioxidative properties. Further work concerning the amelioration of $A\beta_{42}$ induced neurotoxicity, the active ingredients of the extracts and the clinical relevance of these substances still has to be done, but the first results of this study have pointed out some promising plants that are possibly useful in AD therapy.

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ASPALATHUS LINEARIS DECREASES OXIDATIVE STRESS IN *CAENORHABDITIS ELEGANS*

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Rooibos tea is a popular South African tea from *Aspalathus linearis* that is famous for its absence of stimulants such as caffeine and for its antioxidant polyphenolic compounds. Oxidative stress is linked to many aging diseases such as Alzheimer's disease (AD) and diabetes. According to the antioxidant ability of rooibos, it is interesting to investigate rooibos as agent to reduce oxidative stress and for prevention of age-related diseases.
In this study, we showed that rooibos has strong free radical scavenging activities, which was assured by DPPH and superoxide anion assays. Oxidative stress was induced in *C. elegans* by juglone. Pretreatment of *C. elegans* with rooibos extract yielded higher survival rates than in the not be treated control. Furthermore, we measured the intracellular H_2O_2-related level of reactive oxygen species (ROS) in *C. elegans*. The result shows that rooibos treatment reduced ROS level. We also used the transgenic TJ375 (*hsp-16.2/GFP*) strain that expresses the GFP reporter gene in the pharynx in response to the oxidative stress. We found that under the oxidative stress the density of the GFP expression in worms pretreated with rooibos extract is lower than that of worms without treatment.
Those findings suggest that rooibos has beneficial effects by reducing oxidative stress.

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THE ANTIMETASTATIC POTENTIAL OF NOVEL INDIRUBIN DERIVATIVES

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In this current study, we are investigating the influence of 6-bromo-indirubin-3'oxime (6BIO) and 7-bromo-indirubin-3'oxime (7BIO) on induction of apoptosis, cell proliferation, and anti-migratory effects in the well characterized pancreatic carcinoma L3.6pl and breast carcinoma Skbr3 tumor cell lines and characterize underlying mechanisms. 6BIO and 7BIO at doses of 10 µM were shown to significantly reduce the proliferation and viability as well as induce apoptosis in both cell lines. In addition, 6BIO, but not 7BIO, significantly reduced the migration of both cell lines, nearly halting them completely in the Skbr3 wound healing assay at sub-apoptotic doses (3 µM). Chemotaxis was dramatically disrupted and tumor cells significantly lost their ability to invade through membranes or Matrigel™ layers in response to chemoattractants. An increase of the phosphorylation site S785 of β1 integrin is seen upon 6BIO which has been linked to decreased motility of carcinoma cells Additionally adhesion of Skbr3 tumor cells to fibronectin was reduced by 6BIO stimulation. The effects of 6BIO can be attributed to its reduction of the T308 phosphorylation site of Akt, most likely through its direct inhibition of PDK1, ultimately causing long term alterations to the actin cytoskeleton. Erk, FAK and Rac1 levels are unaffected, but cycling of these signaling molecules appears to be disrupted upon treatment.
Finally 6BIO reduced the metabolic capabilities of Skbr3 spheroids at low doses, caused the dissolution of spheroid structures at higher doses and significantly blocked the migration of Skbr3 spheroids. Taken together, the results of this study strongly suggest that the indirubin derivative 6BIO operates by inhibiting different mechanisms in human tumor cells its exert their potent anti-tumor efficacy.

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RFC AND OCT1/2 MRNA EXPRESSION IN UROTHELIAL AND NON-UROTHELIAL BLADDER CARCINOMAS

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Urinary bladder cancer (UBC) ranks ninth in worldwide cancer incidence. The spectrum of bladder tumours includes urothelial (transitional) cell carcinoma (TCC), and non-urothelial carcinoma (squamous cell carcinoma (SCC) and adenocarcinoma). The incidence of UBC varies over the world where TCC is more prevalent and well-studied. In Egypt, however, the pattern of UBC is unique in that both the transitional and squamous cell types prevail. At least 5% of all human genes are transporter-related. Most drug transporters belong to the SLC family of transporters which mediate uptake and chemosensitivity for anticancer drugs. The organic cation transporters (OCT1/SLC22A1 & OCT2/SLC22A2) and the reduced folate carrier (RFC/SLC19A1) are among the most important determinants of chemosensitivity to anticancer drugs. OCT1 and OCT2 transport cisplatin while RFC is the major entry route for anti-folates, whereby platinum-based agents and anti-folates are important components of the chemotherapeutic armamentarium for bladder cancer. Nevertheless, membrane transport of anti-cancer agents, by RFC or OCTs, is considered as limiting to antitumor activity. Quantification of RFC and OCT1/2 in mucosa of 39 untreated bladder cancer patients was performed using real-time quantitative PCR (RT-qPCR) using SYBR Green chemistry. OCT1 and RFC mRNA steady-state levels were found to be ~8.5fold and ~9fold higher, respectively, (N=39; P<0.0001) in the analyzed bladder tumor specimens relative to normal bladder RNA (commercial calibrator, *Clonetech*). RFC upregulation was strongly correlated with tumour type (p<0.05) and, hence, may be considered as a potential diagnostic marker for urothelial versus non-urothelial tumours. However, no significant difference was detected upon comparing OCT1 expression levels among the different tumor types. OCT1 and RFC mRNA levels were not associated with tumour grade or stage. OCT2 mRNA expression was not detected in any of the bladder cancer specimens utilized in this study. The results of this study implicate that both RFC and OCT1 (but not OCT2) may be considered as potential markers for predicting response to chemotherapy in bladder carcinomas.

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THE INSULINOTROPIC EFFECT OF TEA, BUT NOT ITS K^+ CHANNEL-BLOCKING EFFECT, IS DEPENDENT ON GLUCOSE METABOLISM

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Stimulators of insulin secretion depolarize the plasma membrane of the pancreatic B-cell by closure of ATP-dependent K^+ channels. The consequence is the opening of voltage-dependent Ca^{2+} channels (VDCC) which finds its expression as action potential spiking. Action potentials are thus regarded as determinants of the Ca^{2+}-regulated exocytosis of insulin secretory granules. Tetraethylammonium (TEA) is known to increase the B-cell action potential amplitude by block of K_v channels and to increase insulin secretion. Paradoxically, we found that 10 mM TEA reduced the insulin secretion produced by a maximally effective concentration of the sulfonylurea glipizide by more than 50 %. In contrast, the insulinotropic effect of glipizide was enhanced by TEA when a basal glucose concentration was present. In the presence as well as in the absence of glucose glipizide produced a plateau depolarization with superimposed action potentials. Under both conditions, TEA increased the glipizide-induced action potential amplitude by about 50%. The action potential duration was only moderately increased, in contrast to the more than 20fold increase by the opener of L-type Ca^{2+} channels, Bay K8644. Under the same conditions, TEA transformed the plateau-like increase of the cytosolic free calcium concentration ($[Ca^{2+}]_i$) produced by glipizide into a further oscillatory increase. TEA did not negatively affect parameters of B-cell energy metabolism (NAD(P)H fluorescence and ATP/ADP- ratio). So there is a clear dissociation between electric and ionic events on one side and stimulation of secretion on the other side. Apparently, the above sequence of depolarization and Ca^{2+} influx via VDCC is insufficient to stimulate insulin secretion but additionally requires the presence of glucose as a beta cell nutrient to become effective.

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ABC-TRANSPORTER MEDIATED INTERACTION POTENTIAL OF ETRAVIRINE

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Introduction: Etravirine is a novel next generation non-nucleoside reverse transcriptase inhibitor (NNRTI) for the treatment of HIV-1 infections having the advantage of a higher genetic barrier to resistance. ATP-binding cassette (ABC) -transporters are important drug efflux transporters that modulate safety and effectiveness of antiretroviral therapy and have the potential to mediate clinically relevant drug-drug interactions. To increase the understanding of the interaction potential of etravirine we investigated whether etravirine is a substrate, inhibitor, or inducer of important ABC-transporters.
Materials and Methods: We evaluated P-glycoprotein (P-gp/ABCB1) inhibitory potential by calcein assay in P388/dx and L-MDR1 cells. The inhibitory potential on breast cancer resistance protein (BCRP/ABCG2) was assessed by flow cytometric pheophorbide A efflux in MDCKII-BCRP cells. Substrate characteristics were evaluated by growth inhibition assay in MDCKII cells overexpressing human P-gp/ABCB1, BCRP/ABCG2, MRP1/ABCC1, MRP2/ABCC2, or MRP3/ABCC3. Induction of ABC-transporters was quantified by real-time RT-PCR in LS180 cells after four days of treatment.
Results: Etravirine did not inhibit P-gp/ABCB1 in P388/dx and L-MDR1 cells up to the maximum concentration tested (10 µmol/L). In contrast, data demonstrate that etravirine is a potent inhibitor for BCRP/ABCG2 (IC_{50} 1.0 ± 0.4 µmol/L). Growth inhibition assays suggest that etravirine is not transported by the ABC-transporters investigated. At 1 µmol/L etravirine slightly induced expression of *ABCB1* and *ABCC3* and strongly increased expression of *ABCG2* (about 3.5 fold).
Conclusion: Our study indicates that drug transporter mediated interactions with etravirine might evolve particularly at the level of BCRP/ABCG2 for which etravirine is a strong inhibitor and inducer.

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HOW 2B BOUND – NEW INSIGHTS INTO LIGAND RECOGNITION BY THE HUMAN ADENOSINE A_{2B} RECEPTOR

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The human adenosine A_{2B} receptor (hA_{2B}) is one of the four G-protein coupled adenosine receptors. The hA_{2B} receptor has been implicated to play a role e.g. in inflammation, neurodegenerative disorders and tumor growth. However, the receptor's role in (patho)physiological processes is far from being completely understood. In part, this is due to the lack of highly potent and selective ligands, which are also desired because of their therapeutic potential. To gain deeper insights into the structure of the hA_{2B} receptor's binding pocket, and thus obtain more information for the design of the desired ligands, mutagenesis studies were performed. Ten amino acids, predicted by a computer-generated homology model to be in close proximity to the docked ligands, have been exchanged for alanine: Tyr10, Leu81, Asp159, Val169, Asn186, Met198, Trp247, Val250, Ser279 and His280. The receptor mutants were stably expressed in CHO cells using a retroviral vector. For the pharmacological characterization of these mutants cAMP accumulation assays and radioligand binding studies using the new antagonist radioligand [^{3}H]PSB-603 were performed. While the Asp159, Val169 and Met198 mutants showed similar pharmacological profiles as the wild type receptor, Tyr10 seems to be involved in agonist, but not antagonist binding. Asn186 was found to be crucial for antagonist binding, but might even hamper agonist binding. Leu81, Val250 and His280 appear to be important for both antagonist and agonist binding. Trp247 and Ser279 are apparently not involved in antagonist binding, but appear to be crucial for binding of nucleosidic agonists. Using the data of these mutagenesis studies, the hA_{2B} receptor model could be refined, facilitating the rational design of new potent and selective ligands of the hA_{2B} receptor.

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EFFECTS OF ORAL ANTIDIABETICS ON PROLIFERATION AND MIGRATION OF HUMAN CORONARY SMOOTH MUSCLE CELLS

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Objectives: Proliferation and migration processes of vascular smooth muscle cells play crucial roles in the progression of atherosclerosis, stenosis and restenosis after vascular intervention. Diabetes mellitus is known to be a major risk factor for the development of atherosclerosis. In our current study, we have therefore investigated the effects of oral antidiabetics on proliferation and migration of cultured human coronary artery smooth muscle cells (HCASMCs).
Methods: The proliferation experiments were performed in serum-free medium for five days. Changes in impedance were determined online using the xCELLigence System. 24 h prior to the experiment, the medium was replaced by serum-free medium without any supplements. To study migration, HCASMC were seeded onto two-chamber-plates and the rate of migration was determined by measuring changes in impedance in the second chamber. Expression of early genes was determined by real-time RT-PCR.
Results: Addition of the sulfonylurea glyburide (10 µM) caused a marked (49%) inhibition of proliferation. In the presence of diazoxide (0.1 µM), the inhibition was abolished. Rosiglitazone and pioglitazone (0.1 µM) also inhibited cell proliferation. The effects of these peroxisome proliferator-activated receptor (PPAR)γ-agonists were blocked by the potent antagonist T0070907 (2-chloro-5-nitro-N-4-pyridinyl-benzamide). The dipeptidyl-peptidase(DPP)-4 inhibitors vildagliptine and sitagliptine (0.1 µM) also caused a weak inhibition of cell growth. On PDGF (platelet-derived growth factor)-induced migration, glyburide had no significant effect, whereas pioglitazone produced a reduction by 63% and vildagliptine, sitagliptine and saxagliptine increased migration. All tested oral antidiabetics induced the expression of early genes (NR4A1, FOS, EGR1, EGR3).
Conclusion: Closing of K(ATP)-channels by glyburide significantly inhibited the proliferation of cultured coronary smooth muscle cells, but had no effect on migration. PPARγ-agonists inhibited both proliferation and migration. In contrast, DPP-4 inhibitors caused a weak inhibition of proliferation and a strong increase in migration activity. The latter effect may contribute to progression of atherosclerosis.

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PROMISCUOUS SIGNALING PATHWAY ACTIVATION BY OXOTREMORINE M VIA THE MUSCARINIC M_2 ACETYLCHOLINE RECEPTOR IN PRIMARY CELLS

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The muscarinic acetylcholine receptor belongs to the superfamily of G protein-coupled receptors (GPCRs). Stimulation of the M_2 receptor by conventional agonists such as oxotremorine M is known to activate preferentially the $G_{i/o}$ pathway but also G_s pathway activation is detected, an effect called promisquous signaling. Until now, this has only been investigated under non-physiological conditions, i.e. in cells overexpressing the M_2 receptor. To examine G_s pathway activation in primary cells, spontaneously beating murine embryonic atrial cardiomyocytes (CD1, d 14.5-16.5) were used. Beating ceased immediately upon superfusion with a supramaximal concentration of oxotremorine M, suggesting $G_{i/o}$-pathway activation; this effect could be almost completely reversed upon wash-out. In contrast, cardiomyocytes pretreated with the $G_{i/o}$ protein inhibitor PTX (1000 ng/ml for 20 h) responded with an increase in beating rate by 23 ± 5% (means ± s.e.m., n=11) suggesting G_s-pathway activation. To further probe this concept, an allosteric/orthosteric receptor activator, "hybrid 1" (structure shown in [1]), was applied. This compound combines high potency receptor activation via the orthosteric site of the muscarinic M_2 receptor with G_i signaling pathway selectivity. This is probably caused by the interaction with the allosteric receptor site. Hybrid 1 is devoid of Gs activation, even in cells overexpressing the M_2 receptor [1]. The dualsteric agonist suppressed spontaneous beating of the embryonic atrial cardiomyocytes at supramaximal concentrations (which) but did not alter beating rate after PTX incubation. We conclude that promiscuous M_2 receptor signaling is not restricted to cells with artificial M_2 receptor overexpression, but rather a physiological signaling mechanism in intact primary cells.

[1] Antony et al. (2009) FASEB J. 23: 442-450.

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GLUCOSE ACTS OSTEOTOXIC IN ESC DIFFERENTIATION BY ALTERATIONS IN THE WNT SIGNALING PATHWAY

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Diabetes has become a prevalent disease during the last decades. High blood glucose levels caused by this disease are known to affect bone regeneration and turnover. In addition to affecting bone formation in the adult, diabetes in pregnant women is also associated with a higher risk for the fetus to develop skeletal malformations. Since Wnt/beta-catenin (CatnB) signaling was shown before to play important roles in embryonic skeletogenesis, we hypothesized that hyperglycemia may alter bone formation through modulating CatnB activity.
To mimic diabetic hyperglycaemia during embryonic development, we induced differentiation in murine embryonic stem cells (mESCs) in media containing either low (1.0 g/l) or high (4.5 g/l) concentrations of D-glucose. The formation of osteoblasts and osteoclasts, the two cell types found in the skeleton, were evaluated at distinct time points with cell type specific stainings, quantitative PCR and flow cytometry. As these cells are typically derived from the mesoderm, we also checked the expression of typical lineage markers for endo- and ectoderm. Using a LEF/TCF reporter ESC line, the nuclear activation of CatnB was investigated.
Our results indicate that glucose acts as a developmental osteotoxicant in the mESC differentiation model. In particular, diminished osteoblast and osteoclast formation were found in high glucose concentrations, characterized by a 2.6fold decrease in bone specific marker expression. In addition, matrix calcification mediated by the bone forming osteoblast was over 100fold decreased in diabetic conditions. Beside the mesodermal bone cell formation also the development of the ectodermal lineage was decreased in hyperglycaemia (0.55fold), while the commitment of endodermal derived cells was more than 6fold enhanced. Wnt signaling was altered when cells were differentiated in high glucose and showed an accelerated TCF/ CatnB activity. Ultimately, our study gives first evidence that glucose influences skeletal differentiation of mESCs potentially by regulating nuclear CatnB activity. Since CatnB is regulated in early development, it may also affect the differentiation of other germ layers.

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THE INSULINOTROPIC EFFECT OF FLUOROQUINOLONES: MORE THAN ONE MECHANISM OF ACTION INVOLVED

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Several antimicrobial fluoroquinolones induce potentially life-threatening hypoglycaemias by stimulation of insulin secretion. The insulinotropic effect is generally explained by their ability to block K_{ATP} channels in pancreatic beta cells. However, using metabolically intact primary beta cells we have found that fluoroquinolone concentrations which were sufficient to strongly inhibit K_{ATP} channel activity in open cells were unable to significantly depolarize the beta cell plasma membrane. Supposing that an interference with mitochondrial energy metabolism was the reason we investigated the effects of gatifloxacin, ciprofloxacin and moxifloxacin on the NAD(P)H- and FAD-autofluorescence, the mitochondrial membrane potential (Rhodamine 123-fluorescence) and adenine nucleotide content of isolated pancreatic islets or single beta cells. 20 mM glucose induced a NAD(P)H increase of 35 ± 9 % (n =6). This increase was abolished by 100 µM moxifloxacin ($p < 0.01$), whereas ciprofloxacin or gatifloxacin (100 µM each) did not induce significant changes. The FAD-fluorescence decreased by 20 ± 5 % in response to 20 mM glucose (n =4). This glucose-induced change was significantly diminished by moxifloxacin (decrease only by 8 ± 3%, $p < 0.05$) but not by ciprofloxacin or gatifloxacin. Moxifloxacin, but not ciprofloxacin or gatifloxacin significantly reduced the normalized ATP/ADP ratio to 81.5% ($p = 0.0014$, n =7). The hyperpolarizing effect of 20 mM glucose was partially antagonized by moxifloxacin ($p < 0.05$, n =4), but not ciprofloxacin or gatifloxacin. Conclusions: Apparently, fluoroquinolones interact with the respiratory chain and thereby inhibit the energy metabolism of the pancreatic beta cell. The most marked effect is exerted by moxifloxacin and may be sufficient to counteract the K_{ATP} channel blocking effect and/or its consequences for the release of insulin.

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SAFFRON AND TRANS-CROCETIN INHIBIT THE ATP-INDUCED CALCIUM MOBILISATION IN RAT NEUROBLASTOMA CELLS

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Saffron, the dried stigmata of *Crocus sativus* L., is used in traditional medicine for a wide range of indications including cramps, asthma and depressive mood. Studies indicate a neuroprotective potential of saffron extract and several isolated compounds. The underlying mechanisms are not completely known. We found that an ethanolic saffron extract (CSE) and trans-crocetin, a carotinoid from saffron, act antagonistic on NMDA receptors. A current study showed, that trans-crocetin inhibited the ADP-induced platelet aggregation by decreasing intracellular Ca^{2+} release and extracellular Ca^{2+} influx [1]. Mechanisms or receptors which are involved were not characterised.
In this study we examined the influence of CSE and trans-crocetin on the ATP-induced increase of the intracellular calcium concentration ($[Ca^{2+}]_i$) in rat B104 neuroblastoma cells using a FURA-2-imaging system. The P2 receptor agonist ATP caused a concentration dependent (1 nM – 1 mM) increase of the $[Ca^{2+}]_i$ with a transient peak descending to a short plateau phase with a maximum effect by 100 µM. The basal level of Ca^{2+} decreased in Ca^{2+}-free medium containing 1 mM EGTA. The ATP (100 µM) induced Ca^{2+} peak was reduced and the plateau phase was removed under this conditions. This indicates that an initial release of Ca^{2+} from intracellular stores and an influx of extracellular Ca^{2+} are involved in the Ca^{2+} mobilisation.
CSE (100 µg/ml) decreased the ATP-induced increase of the $[Ca^{2+}]_i$ by 33.9 ± 5.8 %. The effect was concentration dependent (10-100 µg/ml) and still remained after a washout period of 15 min. Trans-crocetin 10 µM inhibited the ATP-induced increase of the $[Ca^{2+}]_i$ by 26.1 ± 2.8 %. The effect was also concentration dependent (1-50 µM) but not reversible after a washout period of 15 min. Further investigations are necessary to elucidate the involved mechanisms.

[1] L. Yang et al. 2008 Involvement of Ca^{2+} in the inhibition by crocetin of platelet activity and thrombosis formation. J. Agric. Food Chem. 56:9429-9433

P217

FÖRSTER RESONANCE ENERGY TRANSFER BETWEEN 2´-MANT-3´dGTP AND NITRIC OXIDE SENSITIVE GUANYLYL CYCLASE

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The heterodimeric enzyme NO sensitive guanylyl cyclase (NOsGC) catalyzes the conversion of GTP to the second messenger cGMP. Nitric oxide (NO) activates NOsGC by binding to its prosthetic heme group. (N-methyl)anthraniloyl-substituted nucleotides (MANT-NTPs) are fluorescently labeled nucleotides that compete with GTP or ATP for binding to the substrate binding site of adenylyl and guanylyl cyclases (Gille et al., 2004). In the current work we use MANT-GTP derivatives and Förster resonance energy transfer (FRET) to probe the conformation of the active site of purified NOsGC under basal and NO-stimulated conditions. Using a spectrofluorometer purified NOsGC (3 µM) was excited at 280 nm or 295 nm (tryptophan specific excitation) which led to emission with a maximum at 335 nm. This maximum decreased in the presence of 2'-MANT-3'dGTP (3 µM) and a new peak with a maximum at 430 nm appeared. This indicates that tyrosine (Y) and tryptophan (W) residues serve as FRET donors, while 2'-MANT-3'dGTP acts as FRET acceptor. The FRET efficiency was 12.8 % for excitation at 280 nm and 6 % for the excitation at 295 nm. Therefore almost half of the total energy transfer at 280 nm excitation is caused by tryptophan residues even though the ratio of tryptophan to tyrosine is 5:37. Addition of the NO-donor DEA/NO increased FRET efficiency after excitation at 295 nm. This effect was absent for a mutant isoform of the β_1 subunit which is also unresponsive to DEA/NO in guanylyl cyclase activity measurements. Based on these data we hypothesized that tryptophan residues come closer to the substrate binding site upon activation by NO leading to the observed increase in FRET efficiency. Tryptophan 669 of the rat α_1 subunit is closest to the binding site of GTP as predicted by homology models. Site directed mutagenesis of this residue to alanine led to a decrease in FRET efficiency at 295 nm excitation from 6 % to 4.3 % for rat α_1 W669A. Addition of DEA/NO led to an increase in FRET efficiency as seen before for the wild type enzyme. Thus we were able to show that W669 is partly responsible for FRET, but is not responsible for the increase in FRET-efficiency after addition of DEA/NO. We are currently mutating tryptophan residue W22 in the heme NO binding (HNOB) domain of the β_1 subunit to test whether this residue leads to the increase in FRET efficiency upon activation by NO.

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CYTOTOXICAL AND PHARMACOLOGICAL EFFECTS OF NEW SELECTIVE PDE10A LIGANDS

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Phosphodiesterases (PDEs) are essential regulators of cyclic nucleotide signaling with several physiological functions. Because of their therapeutic importance, PDE inhibitors became distinguished as therapeutic agents in the treatment of various diseases. Phosphodiesterase 10A (PDE10A) was recently identified as a cyclic nucleotide phosphodiesterase expressed primarily in dopaminoreceptive medium spiny neurons in the striatum. Inhibitors of the PDE10A may be interesting for the treatment of neurodegenerative and psychiatric disorders including Alzheimer's disease, schizophrenia and depression. A novel approach for clinical diagnosis are selective PDE10A ligands using as PET ligands. The aim of the present study was to establish cell-based assays to screen cytotoxic and pharmacological effects of fluoric substituted derivatives of a lead compound with high affinity and selectivity for the PDE10A. Cytotoxicity was investigate concentration- (1 nM-100 µM) and time- (12 h-48 h) dependently on human neuroblastoma-(SH-SY5Y), kidney-(HEK293) and hepatocyte- (HEPG2) cell cultures using the cell vitality assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and a lactate dehydrogenase (LDH) assay. Pharmacological effects were determined using functional studies (calcium-imaging) and electrophysiological investigations (intracellular recordings). In vivo toxicity was investigated using the fish embryo toxicity test with zebra danio. The lead compound and the fluoric substituted derivatives had no effect in the MTT and LDH test after short term incubation (12 h and 24 h), but reduced the cell viability and membrane integrity at high concentration (100 µM) after long term incubation (48 h). The lead compound showed no influence on the intracellular calcium concentration, whereas high concentrations (100 µM) of the fluoric substituted derivatives increased the intracellular calcium concentration. Electrophysiological investigations indicated no effect in membrane potential and input resistance of all tested compounds at 100 µM. Using the in vivo test system an acute fish mortality was found after 48 h at 1µM (LC_{50}= 0.1-1 µM). Our results indicate no toxic effect in concentration relevant for PET-ligands, but suggest a different pharmacological profile of the lead compound and fluoric substituted derivatives maybe by district binding characteristics to the PDE10A enzyme.

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ANALYSIS OF HERBAL COMPONENTS CONTRIBUTING TO THE EFFECT OF STW 5 ON RAT COLON

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STW 5 (Iberogast) is a fixed combination of nine herbal extracts used in the therapy of motility-related diseases of the gastrointestinal tract. Its main component is an aqueous-ethanolic fresh plant extract from *Iberis amara* (STW 6). As STW 5 is a combination of herbal extracts, it seems reasonable to study the effects of its herbal components to evaluate the contribution to the effect of the fixed combination.
Studies were conducted with isolated rat colon preparations. STW 5 and its components were tested to influence the basal tone and ACh (100 µM)-induced contractions using isometrical measurement.
Comparable to ileum/ jejenum preparations STW 5 relaxed the basal tone and decreased the ACh-induced contractions in a concentration-dependent manner (64-512 µg/ml). The study of the herbal components containing in STW 5 indicated that peppermint leaf (*Menthae piperitae folium*) had no effect on the basal tone but decreased in low concentration (9.7 µg/ml) the ACh-induced contractions whereas high concentrations (19.4-38.9 µg/ml) did not influence the contractions. STW 6 (3-24.1 µg/ml) as well as chamomilla flowers (*Matricariae flos*, 14.5-116.3 µg/ml) and liquorice root (*Liquiritiae radix*, 10.6-84.9 µg/ml) had no effect neither on the basal tone nor on the ACh-induced contractions.
Our results indicate that the components of STW 5 contribute differently to the spasmolytic and tonicising effects of STW 5 on rat colon preparations.

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IN VITRO STUDY ON THE EFFECT OF AQUEOUS AND ETHANOLIC EXTRACTS OF STW 5 AND STW 6 ON RAT SMALL INTESTINE

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STW 5 is a fixed combination of ethanolic extracts of nine different herbal constituents with a final ethanol concentration of 31 % V/V. Earlier data show an anti-inflammatory and spasmolytic effect of STW 5 in intestinal smooth muscle in vitro. In order to characterize further the effects of STW 5 and its main component STW 6 (Iberis amara) aqueous and ethanolic extracts were compared for their activity to influence the ACh-induced contraction using rat ileum/jejunum preparations. Lyophilisates of the herbal extracts were dissolved either in water or in ethanol (31 % V/V). In the absence of drugs ethanol (final concentration 0.031 % V/V) did not influence the basal tone and the ACh (100 µM)-induced contraction when it was applied directly into the organ bath or after preincubation of the preparation with 0.031 % V/V ethanol for 30 minutes. STW 5 as aqueous or as ethanolic solution inhibited the ACh (100 µM)-induced contractions after application into the organ bath with no significant difference by 12.1±3.2 % and 9.7± 4.5 %, respectively. Under the same condition aqueous STW 6 was without effect on the ACh (100µM)-induced contractions whereas ethanolic STW 6 increased the contractions by 13.9±3.5 %. TNBS (0.01 M) preincubation for 30 minutes resulted in a declined ACh (100 µM) contractions. Using these preparations ethanol (0.031 % V/V) did not affect the reduced contraction. When aqueous or ethanolic extracts of STW 5 and STW 6 were applied to the TNBS preincubated preparations no differences were found in increasing the TNBS-reduced ACh contractions (TNBS 33.7±4,1 %, STW 5 aq. 49.1±4.8 % eth. 58.2±3.4 %, STW 6 aq. 65.7±6.7 % eth. 62.8±5.8 %). The results indicate that the solvent ethanol (1:100) did not influence the effects of STW 5 and STW 6 on intestinal contractility and, therefore, complement the electrophysiological investigations previously published(1).

1) Storr et al. 2004, Digestion 70:257-264

P221

NUCLEAR TRANSLOCATION OF HEME OXYGENASE UNDER CELLULAR STRESS CONDITIONS IS ISOFORM SPECIFIC

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Heme oxygenases (HO's) are potential drug targets in cardiovascular disease. By catalyzing the degradation of the oxidant heme to biliverdin, carbon monoxide and iron, they have protective activities in the setting of inflammatory atherosclerotic diseases. There are two HO-isoforms: the inducible HO-1 and the constitutively expressed form HO-2. Besides the difference in expression no biochemical differences have been detected. The subcellular localization is also thought to be identical as both isoforms have a carboxy-terminal membrane anchor that interacts with the outer membrane of the endoplasmic reticulum. HO-1 is truncated at its carboxy-terminus and translocates to the nucleus after treatment with hemin or under hypoxic conditions (Lin et al, JBC 2007; 282: 20621-20633). It was the goal of the current study to compare the HO-isoforms with respect to subcellular localization and translocation under cellular stress conditions.
Green fluorescent protein (GFP) was fused to the amino-terminus of HO-1 or HO-2 and the resulting fluorescent enzymes were expressed in human embryonic kidney (HEK 293) cells. Analysis using a confocal laser scanning microscope showed endoplasmic reticulum-localization for both isoforms. Deletion of the carboxy-terminal membrane anchor in these GFP-fusion proteins (HO-1 ΔC 267 and HO-2 ΔC 289) led to a nuclear and homogenous cytosolic distribution for both isoforms. This implies that proteolytic cleavage of the carboxy-terminal membrane anchor after cellular stress conditions or other signal transduction events could also lead to nuclear translocation of HO-2.
Exposure to hypoxia (3% O_2) or hemin 100 µM for 48 h led to nuclear translocation and cytosolic redistribution of HO-1 but not HO-2. This implies that the signal transduction pathways that initiate nuclear translocation of HO-1 are at least in part specific for this isoform. It is thought that nuclear HO-1 can activate transcription factors and regulate its own expression (Lin et al, Free Radic Biol Med 2008; 44:847-855). We hypothesize that a similar nuclear translocation of HO-2 may be initiated by a distinct signal transduction pathway and that heme regulatory motifs (Cys-Pro-Phe), which are specific for HO-2 may play a role in this process.

P222

PATIENTS` PREFERENCES FOR WRITTEN INFORMATION ABOUT EFFECTS AND UNDESIRABLE SIDE EFFECTS OF DRUGS

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Package information leaflets (PILs) are often perceived as too long, deterrent and difficult to understand by patients. We thus investigated which information patients desire about their drugs and how this information should be presented. We conducted six focus groups with n = 5-7 patients each with diabetes, arterial hypertension and hypercholesterolemia to elicit their wishes for written drug information. From the interview data, attributes (e.g., prevalence of side effects) and their corresponding levels (e.g., graphical versus numerical presentation of prevalence) were derived by content analysis in a multidisciplinary team including patients. Focus groups revealed that PILs cause emotional responses such as anxiety, uncertainty or dissatisfaction in patients resulting in a broad spectrum of reactions, ranging from seeking for help to non-compliance. Patients demand readable, attractive and short PILs written in simple language with an accentuation of important information by colour or typeface. Six attributes, four relating to content of drug information leaflets and two relating to presentation of information, were identified to be of great importance for patients.
These findings were used in a stated-preference task (discrete choice experiment) in 1000 people aged 50 years and above. 16 scenarios were developed, paired in binary choices and presented to respondents in order to identify the relative importance of each attribute. Patients preferred coloured over black-white leaflets, the provision of a brief summary and general health tips, but no visual presentation of quantification of side effects and no information about what to do in case of side effects. Patients` preferences were dependent on age and education. The elderly preferred less information. Furthermore, they wished more often information in case of side effects, declaration of all side effects and more rarely a brief summary than the younger. Patients with a higher level of education valued the declaration of all side effects and the declaration of information in case of side effects higher than patients with a lower level of education.
These attributes will be tested in a subsequent randomised controlled trial.
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P223

DETECTION OF THE ADENINE RECEPTOR IN HUMAN AND RAT NEURONAL AND NON-NEURONAL CELLS

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Adenine was identified to be an endogenous ligand of a G-protein coupled receptor in rats. There is evidence for its protective effects on neuronal cells. A mouse ortholog but not a human ortholog was described [1]. Preliminary radioligand binding studies using a human neuronal cell line suggest the existence of a human ortholog. The aim of the present study was to detect the adenine receptor on human and rat neuronal as well as non-neuronal cell lines. Human THP-1 and SH-SY5Y cells were used, whereas for rats NR8383 and B104 cells were investigated. The influence of adenine (10 µM to 1 mM) on cell viability and cell death was determined after 12 and 36 hours of incubation using the MTT and LDH test. In rat cells the receptor mRNA was detected via qualitative RT-PCR. Adenine increased the cell viability of THP-1 and SH-SY5Y cells in a concentration dependent manner, up to 132.9±8.7 % and 127.8±17.1 %, respectively. In contrast the cell viability of rat NR8383 and B104 cells was decreased to 81.9±6.8 % and 88.4±7.8 %, respectively after 36 hrs of incubation with 500 µM adenine. PSB-08162 was described as an adenine receptor antagonist [2]. In our experiments it antagonized the effect of adenine in all cell types. Interestingly, PSB-08162 itself reduced the cell viability. In a concentration of 100 µM it decreased the cell viability after 36 hrs in SH-SY5Y and B104 to 80.8±6.9 % and 84.7±14.2 %, respectively. Additionally, an interaction between the adenine and the adenosine A1 receptor was shown on rat but not on human cells. Our results indicate that the adenine receptor is expressed on neuronal and non-neuronal cells from both species. The studies represent for the first time the pharmacological evidence of an adenine receptor in human cells. The pharmacological analysis pointed to a partial agonism of PSB-08162 on human and rat cell lines.

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P224

A NOVEL TOOL FOR PROBING THE ACTIVE STATE OF MUSCARINIC ACETYLCOLINE RECEPTORS

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G protein-coupled receptors (GPCRs) are among the most promising targets for drugs and are therefore in the focus of academic and industrial research. In recent years, increasing evidence for major conformational changes between the active and the inactive receptor states of proteins has emerged [1]. However, in radioligand binding studies receptors are most commonly investigated by probing the protein with orthosteric antagonists instead of agonists because antagonist affinity (K_D) is often more than 100-fold higher than that of agonists.
The oxotremorine-like agonist iperoxo is distinguished by an extremely high potency to affect muscarinic acetylcholine receptors [2]. We prepared the *N*-desmethyl-derivative of iperoxo for commercial radiolabelling with [^{3}H]methyliodide. [^{3}H]iperoxo was used in binding experiments with CHO Flp-In™ cells overexpressing the human muscarinic acetylcholine receptor of the M_2 (hM_2) subtype in both, membrane homogenates (10 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.4, 30 °C) and living cells (Hanks' balanced salt solution with 20 mM HEPES).
In homologous competition binding experiments conducted with membrane homogenates [^{3}H]iperoxo labels both, a high affinity compartment (pK_D = 10), which is likely to represent a G protein-coupled receptor population (20 %), and a low affinity compartment, which might reflect an uncoupled receptor fraction. In living cells [^{3}H]iperoxo binding is monophasic and its affinity is reduced but still rather high (pK_D = 8.7), suggesting that the majority of receptors is not coupled with G proteins due to a high intracellular level of guanylnucleotides.
In summary, the high affinity of this novel radioagonist enables to investigate interaction with muscarinic acetylcholine receptors in living cells and may thus provide unique insight into the receptor biology of active-state GPCRs.

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P225

GPR17- STILL AN ORPHAN RECEPTOR

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GPR17, an orphan G protein-coupled receptor, was described as a molecular target for uracil nucleotides such as UDP, UDP-galactose and UDP-glucose as well as for cysteinyl-leukotrienes [1]. However, in 2009, Maekawa et al. [2] postulated that GPR17 is a ligand-independent, negative regulator for the cysteinyl-leukotriene 1 receptor.
We used 1321N1 astrocytoma cells stably transfected with GPR17 in several different functional assays to probe for activation of GPR17 by uracil nucleotides. We measured GPR17-induced signaling on the level of G protein activation using [^{35}S]GTPγS binding assays, of intracellular messenger using fluorimetric Ca^{2+}-measurements, and whole cell response using real-time measurement of dynamic mass redistribution (DMR; Epic® system). None of these functional assays showed a specific GPR17 activation by the above-mentioned nucleotide agonists. However, GPR17 is activated by a small molecule agonist demonstrating a ligand-dependent signaling.
We conclude that GPR17 is probably still an orphan receptor whose endogenous ligand remains to be identified.
We thank Corning® Life Sciences for their support on the Epic® system.
A. St. is member of the NRW International Graduate School BIOTECH-Pharma.
A. Sp. is member of the DFG-funded GRK 677.
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MECHANISMS OF LPS-INDUCED PROLIFERATION OF BEAS-2B CELLS AND EFFECTS OF GINGER COMPOUNDS ON LPS-PROVOKED IL-8 SECRETION

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OBJECTIVE: We recently demonstrated the proliferative effect of lipopolysaccharides (LPS) on BEAS-2B cells, a human bronchial epithelial cell line. Proliferation is an important aspect of airway remodelling in inflammatory bronchial diseases like asthma. The aim of the present study was to elucidate the intracellular signalling pathways leading to increased proliferation. Furthermore, several compounds of *Zingiber officinale* (ginger) were tested with regard to their effect on LPS-induced secretion of the pro-inflammatory chemokine Interleukin 8, which is increased in plasma of asthmatic patients. In traditional medicine, ginger is used as an anti-inflammatory drug.
MATERIALS AND METHODS: Increased DNA synthesis, i.e. cell proliferation, was measured by an [^{3}H]-thymidine assay. Cells were stimulated with lipopolysaccharides from *P. aeruginosa* in the presence or absence of specific inhibitors of intracellular signalling. IL-8 secretion of LPS-stimulated BEAS-2B cells was measured by ELISA (R&D Systems).
RESULTS: Lipopolysaccharides induce increased cell proliferation in BEAS-2B cells; the effect is diminished by the tyrosine kinase inhibitor Genistein, the JNK inhibitor SP 600125 and the p38 inhibitor SB 202190, but not by the ERK1/2 inhibitor PD 98059. The increased IL-8 secretion of BEAS-2B cells stimulated with LPS is attenuated by volatile ginger oil, the monoterpenoid α-Pinene and the sesquiterpenoid ar-Curcumene, but not by Citral, α-Phellandrene or the pungent constituent [6]-Gingerol and its artificial metabolite [6]-Shogaol.
CONCLUSION: The LPS-induced proliferation of BEAS-2B cells is partly mediated by JNK and p38, but not ERK1/2 MAPK, and tyrosine kinases. The volatile oil from Zingiber officinale and only some of its compounds show anti-inflammatory effects in LPS-stimulated BEAS-2B cells.

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EFFECT OF FREE FATTY ACIDS, LIPOPOLYSACCHARIDES AND BISPHENOL-A ON CYTOKINE SECRETION FROM INS-1 CELLS

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An elevated Free Fatty Acid (FFA) supply augments glucose stimulated insulin secretion. (GSIS) and chronic exposure of saturated FFA, particularly in association with elevated blood glucose levels, can reduce insulin biosynthesis, insulin secretion and induce beta-cell apoptosis. Bisphenol-A (BPA) is a component of plastics that line food and beverage containers. It can leach from products in contact with food and can be detected in urine samples. In vitro studies demonstrated effects of BPA on adipocytes differentiation and glucose transport. It is the aim of this study to show the impact on producing relevant immunological effects in INS-1 cells, a rat pancreatic cell line. INS-1 cells were exposed to FFA (4 µg/ml), LPS (0.4 µg/ml) and BPA (10 µg/ml) for 4 and 12 hours. The secretion of cytokines and chemokines was qualitatively measured by a Proteome Profiler® Array Kit (R&D Systems, ARY 008). Interleukin 6 (IL-6) production was quantified by ELISA (R&D Systems, Quantikine IL-6) after incubating cells for 24 hours with various concentrations of BPA in the presence or absence of estrogen. There is a binding affinity of BPA for the nuclear receptors ER-alpha, ER-beta and the classical/non-classical membrane estrogen receptors as well as the G-Protein-coupled receptor (GPR-30). The existence of the Interleukin-17-Receptor (IL-17-R) was demonstrated by using Western-Blot. The Proteome Profiler showed an up-regulation ICAM-1, IL-3, -4, IP-10, MIP-1α after FFA (4 µg/ml) exposure for 4 hours; LPS (0.4 µg/ml)/4 hours: ICAM-1, IFN-γ, IL-1, -4, -6, -10, MIP-1α; combination of FFA (4 µg/ml) and LPS (0.4 µg/ml): ICAM-1, IFN-γ, IL-1, -2, -3, -4, -6, -10, -13, -17 and TNF-α. A 24 h incubation of BPA (10 µg/ml) induced the release of ICAM-1, IFN-γ, IL-4, -6, -10, and -13. An up-regulation (30.5%) of pro-inflammatory IL-6 secretion was observed. The combination of BPA (10 µg/ml) and estrogen (10 µg/ml) demonstrated an increase of IL-6 secretion up to 42.9%; the effect of BPA and estrogen was additive. The results implicate that FFA, LPS and BPA trigger secretion of pro-inflammatory and anti-inflammatory cytokines in insulin secreting cells.

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EFFECTS OF STW 5 AND STW 6 ON RAT ILEAL AND COLONIC PREPARATIONS: A COMPARATIVE STUDY

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The multi-herbal drug STW 5 (Iberogast®) is successfully used for the treatment of gastro-intestinal disorders, like functional dyspesia or irritable bowel syndrome. It is a fixed combination of nine plant extract with *Iberis amara* (STW 6) as its main component.
In this study the influence of STW 5 and STW 6 on tonus and on acetylcholine (ACh)-induced contractions was examined in-vitro to analyze region specific differences of the phytomedicine. We used 1-1.5 cm long untreated and inflamed ileum and colon preparations of male Wistar rats. The inflammation was induced by intraluminal installation of 2,4,6-trinitrobenzene sulfonic acid (TNBS, 10 mM). STW 5 (128-512 µg/ml) concentration dependently reduced the tonus and decreased ACh-induced contractions of untreated ileal and colonic preparations. STW 6 in equivalent concentrations (3-24,1 µg/ml) neither affects tonus nor contractility. TNBS-induced inflammation leads to a significant reduction of ACh-induced contractions. Co-incubation with STW 5 (512 µg/ml) and STW 6 (24,1 µg/ml) partially normalized the TNBS-induced decrease in ACh-induced contractions of ileum preparations. In inflamed colon segments only the co-incubation with STW 6 (24,1 µg/ml) enhanced the ACh-induced contractions, while STW 5 (512 µg/ml) had no effect.
In conclusion, STW 5 influences intestinal motility and tonus, whereas STW 6 does not contribute to these effects. In TNBS-inflamed ileum preparations STW 5 as well as STW 6 normalized the reduced ACh-induced contractions, while in colon preparations STW 6 but not STW 5 is effective. Our study confirm region specific efficacy of STW 5 and its main component STW 6.

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EFFECT OF A_{2B} ADENOSINE RECEPTORS ON RAT TRACHEA TONUS AND ON CILIARY BEAT FREQUENCY

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It was shown that the activation of A_{2B} adenosine receptors induces a relaxing effect on the musculus trachealis of guinea pigs, while activation of the A_1 adenosine receptor mediates a contraction.The nonselective adenosine receptor agonist adenosine-5'-N-ethylcarboxamide (NECA) produces a relaxing but no contracting effect, although it has a higher affinity for the A_1 than the A_{2B} adenosine receptor. The aim was to identify the relevance of A_1 and A_{2B} receptors by using NECA, the selective A_{2B} receptor agonist BAY 60-6583 and the A_{2B} receptor antagonist PSB-1115 on rat trachea contraction and on CBF (ciliary beat frequency). NECA (1.3 and 4.3 µM) induced a contraction (+25.39 % and +61.01 %, respectively) of a trachea precontracted by carbachol. This effect was mediated by A_1 receptors, since the selective A_1 adenosine receptor agonist N^6-Cyclohexyladenosin (CHA, 1 µM) mimicked this effect (+18.02 %). The effect vanished after repeated administrations of NECA (desensitisation) and even a relaxing effect was found by 4.3 µM (-25,86 %). This relaxing effect could be blocked by 5.3 µM PSB-1115 (-6.70 %). BAY 60-6583 was not effective probably due to solubility problems. BAY 60-6583 led to a significant increase of CBF by 12.14 %, which was neutralized by PSB-1115 (5.3 µM). This study shows, that the relaxation on the rat musculus trachealis depends on the activation of the A_{2B} adenosine receptor, whereas the activation of the A_1 adenosine receptor leads to a contraction. A_{2B} adenosine receptors are involved in CBF modulation.
We thank Dr. T. Krahn, Bayer Schering Pharma AG, for providing BAY 60-6583 and Prof. Dr. C. E. Müller, Pharmazeutische Chemie I, Rheinische Friedrich-Wilhelms-Universität Bonn, for supplying PSB-1115.

Poster Pharmaziegeschichte

G230

Traditional plant remedies against fever – potential modern phytotherapeutics?

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Throughout the ages only the symptom “fever”, but not the true etiological causes of the diseases were known. So long before modern pathogenesis and antibiotics changed our understanding of illnesses many different pathological conditions and even full groups of illnesses were called fever. For centuries plants represented the major part of the traditional materia medica and many were used against fever of different origin. A screening of medicinal plants used against fever for centuries and analyzing possible effects as treatment against respective types of fever may give precious hints for special efficacies of the used plants or even their constituents, for example antipyretic, antibiotic or antiviral properties. In this context it is especially important to understand and evaluate the use of the plants on the base of the contemporary, medical-pharmaceutical paradigm.
In the early modern time, for example, particularly plants which contained bitter substances were used against fever. Bitter substances are known to activate the digestion and in those days fever was often understood as a digestion procedure which helps the body to deposit of harmful substances and to restore the balance of the bodily humors. Furthermore bitter plants were described as being “cold” and seemed therefore right to heal a warm condition such as fever. A representative for those bitter plants used as febrifuge is centaury (Centaurium erythraea), also known as “the fever herb”. Dioscurides (1st century) was the first who described its application against fever and named it “Febrifuga”. The authors of the “Kräuterbücher” such as Hieronymus Bock (1498–1554) praised it as remedy against fever as well and in the 19th century centaury was even used as a cheap alternative for the China bark (Cinchona sp.). In 1991 researchers from Turkey actually proved antipyretic and anti-inflammatory effects of an extract of centaury in animals.
Purpose of our work is to prove the continuous use of traditional herbs against fever from the early modern time to today and suggest selected plants for further investigations concerning constituents with antipyretic and antiinfective activity.

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PLANT REMEDIES FOR THE TREATMENT OF WOUNDS FROM EARLY MODERN TIME TO THE PRESENT

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Plant remedies for the treatment of wounds look back on a long tradition in pharmacy. The empirical use for often hundreds of years can be interpreted as an indication for a potential effect on the wound-healing process. Many of these plants fell in oblivion or have experienced a different application. Discovering or rediscovering such plants can be interesting for the development of modern phytotherapeutics for wound treatment. In historical sources of the early modern time like herbals, pharmacopoeias and pharmaco-botanical works far more than hundred herbal medications for wound treatment are mentioned. After the screening of these plants, the medico-pharmaceutical tradition of selected medicinal plants will be analyzed to investigate their stringent application as wound healing agents from early modern time to the present. In combination with the latest scientific data concerning their constituents and efficacy their possible potential for modern wound treatment will be evaluated.

G232

HERBARIUM SIEGESBECKIANUM

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On Lorenz Heister`s recommendation the german physician Johann Georg Siegesbeck (1686-1755), who founded his career as a botanist in Helmstedt, became director of the Medical Garden of Saint Petersburg in 1735. He is well known due to his hassle with Carl von Linné. While establishing his ideas of classifying plants after their sexual organs Linné has been attacked by Siegesbeck. He negated this concept as well for religious as for botanical reasons. The religious aspect of this conflict mainly defines the perception of Siegesbeck in history of science ever since. In 1747 he was excluded from The Saint Petersburg Academy of Sciences for political reasons and returned to Germany to work at Seehausen as a physician.
This poster should highlight the botanical significance of Siegesbeck through his work on the herbarium. His private herbarium came to the Herzog August Bibliothek at Wolfenbüttel and is still stored there today. The circumstances for this transfer to Wolfenbüttel are not known. It contains approximately 1.500 dried specimen in fifteen volumes. Most of the plants are named in pre-linnéan nomenclature and listed in alphabetical order in an added index. The sheets with the glued samples are not fixed to the book and granted Siegesbeck the flexibility to change the order of his herbarium. This order still remains predominantly. A remarkable amount of plants originates from Siegesbeck’s period in Russia and led the Herzog August Bibliothek to date the herbarium to 1735.

REFORMS OF THE PHARMACEUTICAL SYSTEM SHOWN BY THE EXAMPLE OF THE FORMER RHINE PROVINCE (1791-1875)

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The causes for competitive conflicts between doctors and non-legitimised healers are manifold. What fuelled the conflict at the beginning of the 19th was the occupation by the French. When French Patent Law of 1791 was introduced the ancient battle about the division of the pharmaceutical market was sparked off again. In the ensuing Prussian reform efforts, they maintained the subordinated concept of French Patent Law. Especially against the background of tough financial conditions, there was a noticeable increase in outside pressures to reform the pharmaceutical market with respect to the efficiency and costs of therapeutic agents. This is how popular medicine made its official arrival on the remedy scene. Patent laws and privileges existed to protect inventors; at the same time government regulations had to be established to protect the general public from the numerous inventions. The side effects of widespread quackery trade on the so called salus aegroti in turn, influenced the pharmaceutical needs as a regulative factor.

Fachgruppensymposien

F1-1

FÖRDERUNG DER KOOPERATION VON ARZT UND APOTHEKER ALS THEMA DES NEUEN AKTIONSPLANS DES BUNDESMINISTERIUMS FÜR GESUNDHEIT ZUR VERBESSERUNG DER ARZNEIMITTELTHERAPIESICHERHEIT (AMTS)

Dr. Amin-Farid Aly
Arzneimittelkommission der deutschen Ärzteschaft

Der Sachverständigenrat zur Begutachtung der Entwicklung im Gesundheitswesen hat mit seinem Sondergutachten 2009 zum Thema „Generationenspezifische Gesundheitsversorgung in einer Gesellschaft des längeren Lebens“ ausdrücklich darauf hingewiesen, dass in Zukunft in der Arzneimittelversorgung die interdisziplinäre Zusammenarbeit zwischen Ärzteschaft und Apothekerschaft an Bedeutung gewinnen wird. Als wesentliches Ziel wird in diesem Zusammenhang „die Förderung von Therapiesicherheit und bestimmungsgemäßem Gebrauch“ von Arzneimitteln genannt. Trotz vielfältiger punktueller Erfahrungen in der Praxis ist eine zwischen Ärzteschaft und Apothekerschaft abgestimmte gemeinsame Position zur Verantwortung der jeweiligen Bereiche und ihrer Zusammenarbeit zur Verbesserung
der AMTS bislang nicht formuliert worden.

Der Aktionsplan AMTS 2010 – 2012 nimmt die Zusammenarbeit zwischen Apothekern und Ärzten im Bereich AMTS als ein zentrales Thema auf. In der Koordinierungsgruppe die für die Umsetzung und Weiterentwicklung des Aktionsplan AMTS verantwortlich ist, sind sowohl Ärzte als auch Apotheker vertreten. Daher sollen hier Vorschläge für eine Optimierung der Zusammenarbeit zwischen von Ärzteschaft und Apothekerschaft zur Verbesserung der AMTS erarbeitet werden.

Der Vortrag benennt Felder der Zusammenarbeit zwischen Apothekern und Ärzten und zeigt erste Vorstellungen einer möglichen Zusammenarbeit hinsichtlich der Arzneimitteltherapiesicherheit.

F1-2

ZUKUNFT eMEDIKATION. WIE IT DIE PHARMAZEUTISCHE BETREUUNG UNTERSTÜTZEN KANN

Dr. Stefan Schwenzer
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Die durchgehende EDV-gestützte Begleitung und Unterstützung der Medikationsprozesse sowohl in der ambulanten als auch in der stationären Versorgung (kurz eMedikation) ist trotz einzelner Rückschläge (eGK) dabei sich als fester Bestandteil der Arzneimittelversorgung zu etablieren. Auch die pharmazeutische Betreuung wird in Zukunft fester Bestandteil innovativer Versorgungskonzepte sein. Dabei wird sich ein Augenmerk auf eine strukturierte Erfassung und Bewertung der Patientenmedikation richten. Hierbei sollten neben der Medikation möglichst viele Begleitparameter, wie z.B. Alter, Allergien, Diagnosen und Laborwerte berücksichtigt werden. Moderne Softwaresysteme sollen die Bewertung dieser komplexen Zusammenhänge unterstützen und dabei Apotheker und Ärzte mit Informationen versorgen, die über eine einfache Interaktionsprüfung deutlich hinausgehen.
Hier haben Softwarehersteller in den letzten Jahren die Entwicklung deutlich vorangetrieben. Die Kombination moderner IT-Technologien mit semantischer und regelbasierter Wissensanalyse wird inzwischen erfolgreich in der Routine eingesetzt. Dieses unterstützt sowohl eine strukturierte Medikationsdokumentation, als auch die Prüfung der Medikation im Kontext von Diagnosen und Labordaten. Der Einsatz von Services und Webkomponenten ermöglicht dabei die Entwicklung skalierbarer und modularer Lösungen, die sich für eine integrierte Nutzung in der sektorenübergreifenden Versorgung und pharmazeutischen Betreuung anbieten.

F1-3

ERSCHLIEßUNG VON SICHERHEITS- UND WIRTSCHAFTLICHKEITSRESERVEN DURCH DIE DOKUMENTATION ARZNEIMITTELBEZOGENER PROBLEME

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Durch das Erkennen, Vorbeugen und Lösen arzneimittelbezogener Probleme in der Arztpraxis, im Klinik- und Heimalltag aber vor allem bei der Abgabe von Medikamenten in der Apotheke werden die Arzneimitteltherapiesicherheit und die Therapieeffizienz erhöht und mögliche Folgekosten gesenkt. Dies ist durch zahlreiche Betreuungsstudien im Sinne einer leitliniengerechten Versorgung belegt worden. Erkannte arzneimittelbezogene Probleme können mit Hilfe einer standardisierten Klassifikationssystems softwaregestützt dokumentiert werden, wodurch eine patientenindividuelle und arzneimittelspezifische Rückverfolgung, Interaktion und Vermeidung möglich wird. Damit wird auch einer Forderung der WHO entsprochen, die nicht nur die Erfassung unerwünschter Arzneimittelwirkungen sondern auch aller anderen arzneimittelbezogenen Probleme fordert.
Eine systematische und möglichst lückenlose Dokumentation der Arzneimittelanwendung schafft für den Patienten eine individuelle Datenbasis, mit der die Sicherheit der Arzneimitteltherapie erhöht und die Nachhaltigkeit der ärztlich veranlassten Therapie unterstützt werden kann.
Sachlogisch ergeben sich kostenrelevante Nutzenkomponenten einer Arzneimitteldokumentation aus folgenden Einzelschritten, bei denen grundsätzlich auf die gespeicherte Medikationshistorie zurückgegriffen werden muß:

- Berücksichtigung bestehender Kontraindikationen (Allergien, Begleiterkrankungen etc.)
- Vermeidung versehentlicher Fehlverordnungen durch Abgleich mit zuvor verordneten Arzneimitteln, einschliesslich unzweckmässiger Stärken und Darreichungsformen
- Erkennung und Überprüfung von Doppelverordnungen
- Einschätzung der Patientencompliance über die Abstände der Rezepteinlösung
- Überprüfung der individuellen Dosierung (soweit angegeben oder vom Patienten erfragbar)
- Erkennung und Vermeidung schwerwiegender Interaktionen mit klinischen Konsequezen
- Aufnahme von AM-Unverträglichkeiten, die zum Medikationsabbruch geführt haben, als Patientenmerkmal, um eine künftige Wiederverordnung zu vermeiden.

Durch die Klassifizierung arzneimittelbezogener Probleme wird die Kooperation der Heilberufler erleichtert und kann besser auf therapierelevante Schwerpunkte fokussiert werden. Durch einen entsprechenden Dokumentationsstandard für arzneimittelbezogene Probleme soll eine weitere Verbesserung der Arzneimitteltherapiesicherheit bei gleichzeitiger Erschließung von Wirtschaftlichkeitsreserven, etwa durch gezielte Förderung der Compliance erreicht werden.

F2-1

TRACING CHEATERS IN SPORTS – MASS SPECTROMETRY IN ANTI-DOPING RESEARCH

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In sports the (mis-)use of drugs is regulated by the World Anti-Doping Agency (WADA). Its list of prohibited substances classifies the use of several classes of substances and prohibited methods in sports as doping (Tab. 1). The analyses in doping control are mainly performed utilizing GC-MS(/MS), LC-MS/MS and isotope ratio mass spectrometry (IRMS).
Current research mainly focuses on the identification of metabolites suitable for elongated detection of classical doping agents, the discrimination between endogenously produced and synthetic congeners, the detection of new performance enhancing substances and the identification of their metabolites. This also includes the characterization of new designer steroids that are marketed as dietary supplements. Additionally the manipulation of doping control samples is detectable by the help of mass spectrometric techniques.

Tab 1: Classes of prohibited substances and methods in sports

Anabolic Agents	Peptide Hormones, Growth Factors and and related Substances	Beta-2 Agonists	Hormone Antagonists and Modulators	Diuretics and other Masking Agents
Stimulants	Narcotics	Cannabinoids	Glucocorticoids	
Enhancement of Oxygen Transfer	Chemical and Physical Manipulation	Gene Doping	Alcohol *)	Beta-Blockers *)

*) only prohibited in particular sports

F2-3

PREPARATION OF MONOLITHIC COLUMNS FOR LC-MS/MS ANALYSIS OF PROTEINS AND DRUGS

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During the past few years monolithic supports have been used for an increasing variety of applications. The examples for applications of monoliths presented show that the chromatographic performance of bioreactors and affinity media prepared from monolithic media is superior compared to that of conventional particle-based systems. The ease of fabrication and modification combined with a long life time of the prepared columns and their potential to be used in fully automated analytical systems make them attractive tools for yet an increasing number of applications.

F2-4

CE IN PHARMACEUTICAL ANALYSIS – APPLICATION TO DRUG IMPURITY PROFILING

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CE has been recognized as a suitable technique for the determination of the stereoisomeric purity as well as for the analysis of related substances of drugs, for the determination of organic and inorganic counterions, for the analysis of peptides and proteins, etc. [1]. This is also reflected by the fact that CE has been included as a general monograph in the European Pharmacopoeia as well as the United States Pharmacopeia several years ago. The technique is applied in several monographs of the pharmacopeias for drug identification and/or tests.

Traditionally, the stereoisomeric composition of a drug is determined by optical rotation which is not very accurate. Thus, current research efforts aim at the development of new CE methods for the determination of the stereoisomer composition of drugs. The studies include methods for the simultaneous determination of related substances of the drugs besides the stereoisomers. Such analyses are typically performed in separate tests in the pharmacopeias. Examples for the method development in cyclodextrin-mediated separations in EKC and MEEKC will be discussed.

[1] Capillary Electrophoresis Methods for Pharmaceutical Analysis, S. Ahuja, M. I. Jimidar, eds., Elsevier, Amsterdam, The Netherlands 2008.

F2-5

CAPILLARY ELECTROPHORESIS (CE) AND FRET AS TOOLS FOR TESTING INHIBITORS OF HUMAN PROTEINKINASE CK2

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Protein kinase CK2 is of increasing impact as a target for treating neoplastic diseases. Upregulated CK2-activity can be found in a variety of tumors. Until now, the common *in vitro* assay to quantify compound-driven CK2-inhibition needs the use of radioactive isotopes. We present two novel CK2-inhibition assays that could complete or even replace the standard radiometric assay.
The first assay is based on Förster-resonance-energy-transfer (FRET) between the donor-fluorophor EDANS and the acceptor DABCYL within the CK2 substrate peptide [DABCYL]-RRRDDDSDDD-[EDANS]. This peptide possesses an elastase cleavage site adjacent to the phosphate-acceptor serine. The non-phosphorylated peptide can be cleaved by elastase and consequently FRET is hampered. Upon phosphorylation the elastase recognition site within the peptide is masked, cannot be cleaved and FRET is retained. The degree of phosphorylation is measured as donor-fluorescence intensity that develops during the loss of FRET. Thus fluorescence intensity is inversely correlated with CK2-activity.
The second assay is based on a direct product quantification of a CK2-reaction by capillary electrophoresis. The acquisition of a phosphate moiety leads to a difference in electric charge between substrate and product and enables their electrophoretic separation (“mobility-shift”). Quantification is performed by calculating the area of the product peptide peaks, recorded by UV-absorption. The IC_{50}-values of Emodin and TBB that were determined by this assay showed a good agreement with published data [1]. Subsequently, new inhibitors of human CK2 with IC_{50} values in the nanomolar range could be identified.

[1] Gratz A, Götz C, Jose J (2010) *Electrophoresis* 31:634-40.

F4-1

A YEAR CPOE - PRACTICAL EXPERIENCES AND PERSPECTIVES FROM A CLINICAL PHARMACIST

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Objectives: The use of electronic prescription software increases drug safety in hospital. However, the implementation rate of CPOE in german hospitals is less than 3 percent. Prescription errors occur often at cost-intensive drugs.

Setting: Since December 2008, the hospital uses Rp-Doc® first in the heart-lung vascular center (HLG, 4 wards, 144 beds). Experience with process design as well as clinical and pharmaceutical aspects are presented.

Results: Drug profiles of 50 patients were evaluated. The average number of inpatient prescribed drugs was 7,2 ± 3,28 (1-16). In 82% of the patients was considered a polymedication (more than 4 drugs) and in 32% a renal insufficiency (GFR <50 ml/min). The investment in the HLG-center of 41 T € standing drug-cost savings of 71 T € (about 15%) against.

Conclusions: The introduction of CPOE leads to a modification of workflows. Each change/enter of pharmacy or nursing needs the physician release. Patient safety and quality indicators can be improved. In addition, economic aspects such as cost unit accounting and supplementary reimbursement (Zusatzentgelt) control can be implemented easily.

F4-2

SAFETY OF PHARMACOTHERAPY ON THE INTERFACE BETWEEN OUTPATIENT AND INPATIENT TREATMENT

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Abstract:
Healthcare in FRG primarily takes place on two strictly separated sections, the outpatient and inpatient treatment. Whenever patients with long-term medications (e.g. chronic diseases) need an inpatient treatment in the hospital, a lot of problems and questions in the context of medication occur on this interface, because in German hospitals exist mainly clearly drug formularies and standardised drug therapies. This is the result of cooperation between hospital pharmacists and physicians in the formulary committee. However in the outpatient section drug therapies are only rudimentarily standardised and a plenty of proprietary medicinal products in FRG are available. Unfortunately the existing rebate-contracts between generic manufacturers and statutory insurance companies (GKV) increase the complexity in this sector.
Now for over 15 years, the clinical pharmacist's daily task in our hospital is to ensure the safety of pharmacotherapy when patients with chronic medication enter the surgical wards. Directly on bed-side or in patients chart the previous (individual) outpatient medication is checked and proved. In particular, the plausibility, correct dosage, drug-drug-interaction and specific contraindications are clarified. Recommendations for a drug conversion to our formulary medication and standardised drug therapies are given and are highly accepted by the physicians.
Most recently, the reason for the actual hospital admission (initial diagnosis) is matched with published and documented adverse effects of the previous medication, because sometimes direct or hidden drug adverse effects induce hospital admission.
In this presentation our daily realisation and really happened practical examples are shown.

F4-3

CLINICAL-PHARMACEUTICAL INTERVENTION STUDIES TO OPTIMISE PATIENT SAFETY IN DRUG THERAPY IN HOSPITAL SETTINGS

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Objectives
Drug-related problems (DRP) are frequent in hospitals. Particularly, they occur in prescription and administration and lead to death, prolonged hospital stay, and severe adverse drug reactions (ADRs). Different reasons, such as knowledge deficits, account for DRPs. In a setting of limited resources, however, intervention strategies to improve patient safety have to be prioritised to the most frequent and severe DRPs and have to be tailored to the causes.
Participants and methods
At a university hospital, we identified DRPs in routine care by instructed monitors (clinical pharmacists) and assessed knowledge deficits by a questionnaire survey. DRPs were classified according to their prevalence, potential risk, and reasons for their occurrence using a decision-matrix model. Tailored interventions such as teaching sessions, clinical pharmacist interventions, and newly developed clinical decision support systems were implemented in hospital settings including e.g. intensive care units, paediatric wards, or cancer patients. Relative risk reduction, p-values, and total numbers (control and intervention group) are presented.
Results
In drug administration, we identified physico-chemical incompatibilities and drug administration by gastric tube as most prevalent DRPs (n=1376 processes). DRP prevalence decreased by 59% (p=0.003, incompatibilities, adult ICU patients, n=1108 drug pairs) and by 94% (p<0.001, gastric tube administration in children, n=1164 processes). In a follow-up analysis these effects were sustainable. In drug prescription, guideline adherence in pain treatment for cancer patients increased by 81% (p<0.001, n=100 patients), ADRs caused by drug interactions decreased by 43% (p=0.001, n=265 patients) and excessive dosing in patients with renal insufficiency by 51% (p<0.001, n=68).
Conclusions
Tailored interventions in routine care by clinical pharmacists based on teaching sessions and newly developed electronic systems decreased rates of DRPs and DRP-related ADRs.

F4-4

MEDICATION SAFETY – HOW DO NURSES CONTRIBUTE?

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Background
In Germany several people are involved in the medication process, which starts with the prescription and ends after the drug reaches the place of drug metabolism with the monitoring process. Health professionals involved in the process are nearly always the physician (prescription), the pharmacist (dispenser) and/or nurses; however non health professionals (patients) are also always involved as well as family members sometimes. Good communication is necessary between all persons involved to assure medication safety, as poor communication is associated with medication-related errors and adverse effects. Nurses are usually called on as soon as patients are not capable of managing certain steps within the whole medication process – this applies both to the hospital and the primary care setting. Especially within the long-term care of patients with chronic diseases nurses are often integrated and challenged.
Questions
1. For which tasks within the medication process do nurses in Germany take over responsibility?
2. How do nurses contribute to medication safety?
Answers
Within the hospital setting the nurses' main focus of attention is to survey administration, dispensation of medicines as well as to observe medication effects and side effects in order to reduce medication related problems and medication errors. Employing specially trained pain and/or wound nurses ensures medication (and patient) safety.
In the primary care setting nurses support patient self management, by identifying problems in coping with (complex) medication regimens in everyday life, by supporting patients to manage these and by developing individual medication routines. This raises adherence and hence medication safety.
Nurses often take over communication about medication related issues at the interface between patient, family members and other health professionals and therefore contribute to medication safety. To ensure medication safety a team approach, including all involved health professionals, is necessary.

F5-1

MEDICATION SAFETY OF ELDERLY PATIENTS IN NURSING HOMES

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The multimorbidity of elderly patients often leads to polymedication. Therefore, these patients are at particular risk to suffer from drug-related problems such as adverse drug events. Studies have shown that the incidence of adverse drug events is particularly high in long-term care facilities leading to severe consequences such as hospitalization and death (Gurwitz et al. 2005).
In a prospectively designed cross-section analysis in North Rhine-Westfalia funded by the Federal Ministry of Health (BMG) two clinical pharmacists surveyed the documentation of 789 nursing home residents and interviewed the nurses regarding symptoms observed in the previous month. The incidence of adverse drug events was found to be 8.11 per 100 resident-months with a preventability rate of 57.8%. The majority of adverse drug events were caused by CNS and cardiovascular drugs (Schröder et al., this conference).
Based on these data, a structured intervention was planned by a multiprofessional panel of experts consisting of clinical pharmacologists, clinical pharmacists, general practitioners, nurses, and geriatricians. The intervention comprised five measures: (1) intensive seminars for nurses and pharmacists, (2) advanced training for the prescribing general practitioners, (3) the implementation of a reminder card summarizing high-risk drugs and monitoring issues, (4) the formation of medication safety teams in each nursing home consisting of a nurse and a pharmacist, and (5) structured documentation and communication regarding individual drug therapy. The intervention has recently been implemented in four nursing homes in North Rhine-Westphalia.
In conclusion, incidence and severity of adverse drug events indicate serious deficiencies in the health care of elderly patients living in nursing homes. Medication safety-enhancing interventions have the potential to improve the health status of the residents and to reduce costs, e.g. by avoiding unnecessary falls and hospitalization. The developed intervention is easy to implement and may serve as a model for German nursing homes.
Reference:
Gurwitz et al. Am J Med. 2005;118:251-8

F5-2

DATA DRIVEN QUALITY IMPROVEMENT IN PRIMARY CARE (DQIP): USING ROUTINE DATA TO IMPROVE THE QUALITY AND SAFETY OF PRESCRIBING IN PRIMARY CARE

Tobias Dreischulte

Background
A number of systematic reviews have demonstrated that approximately 4-5% of all unplanned hospital admissions are caused by preventable adverse drug events, of which more than half have been attributed to errors in medication prescribing and monitoring. The 'DQIP' study is a five year research programme which aims to develop and test a complex intervention to improve the quality and safety of prescribing in UK primary care. A central component of this intervention is the analysis (audit) and feedback of providers' achievements against quality and safety indicators based on morbidity linked prescribing data, which has become routinely available as a by-product of novel contractual arrangements between the UK National Health Service (NHS) and general medical practitioners (GPs).

Methods
The design and evaluation of the 'DQIP' intervention follows the Medical Research Council's (MRC) guidance and uses a mix of quantitative and qualitative research methods in six studies divided into two phases. In phase 1 potential quality and safety improvement targets (primary outcomes) are determined (study 1) and tested (study 2), intervention components are piloted (study 3) and options for embedding the intervention into the wider health care environment are explored (study 4). Phase 2 includes the conduct of a cluster randomised trial in two Scottish health boards (study 5) and a parallel process evaluation (study 6) in order to understand how the intervention mediates effectiveness.

Results
The findings of the completed study 1 will be reported, where a broad set of prescribing quality and safety indicators was developed and prioritised based on literature review and expert consensus. Instruments of data feedback that are currently undergoing pilot testing will be presented.
Discussion and conclusion
Opportunities, limitations and methodological challenges of using indicators of prescribing quality and safety in the context of quality judgement, quality improvement and as outcome measures in quality improvement research will be discussed.

F5-4

MORE THAN GOOD PRICES - PATIENT SAFETY IN DRUG THERAPY WITHIN A LARGE COLLABORATION OF COMMUNITY PHARMACIES

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Background: Since the 1990s especially professional bodies proclaimed a change of the pharmacy profession from product centeredness towards patient orientation. Patient centeredness has been associated with the term "pharmaceutical care" and the introduction of the subject "Clinical Pharmacy" into the pharmacy state curriculum. Nevertheless, change towards pharmaceutical care in pharmacies has been slow and not sustainable, e.g. due to limited resources and lack of reimbursement. Notwithstanding, pharmacies might relevantly promote patient safety in drug therapy. So far, data characterising the impact of pharmacies' everyday activities on patient safety in drug therapy have been scarce.
Objectives: To identify a research framework to sustainably establish and evaluate new patient oriented services which promote patient safety in drug therapy.
Setting & Methods: Pharmacies in Germany belonging to the pharmacy collaboration "LINDA Apotheken" were included in this project. We employed quantitative (online survey, register) as well as qualitative (semi-structured interviews, observation) research methods.
Results: Action research was identified as a suitable framework. It is a democratic and participant oriented approach. It consists of the following steps constituting the so called "action research cycle": plan, act, observe and reflect. The first cycle dealt with an online survey (response rate: 30%, 398 out of 1320) on patient oriented services in pharmacies and decision-making about the prospective project: It showed that 98% of all pharmacies use an automated drug-drug interaction (DDI) check. Furthermore, ca. 50% of all pharmacies had some form of documentation of their DDI management. The second cycle, which is ongoing, deals with a pilot study in nine pharmacies investigating the feasibility of an electronic documentation system for the management of DDIs.
Conclusions: Action research might be a promising way towards sustainably establishing and evaluating patient oriented services in community pharmacies, which increase patient safety in drug therapy. First data collected from the participating pharmacies demonstrated a promising basis for the establishment of such services.

F6-1

IN-VITRO MODELS FOR CONGENITAL KERATINIZATION DISORDERS

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Hereditary keratinization disorders are a clinically and genetically heterogeneous group of skin diseases. They are characterized by abnormalities in terminal keratinocyte differentiation. Clinical features include marked keratosis, more or less intense scaling of the skin, either generalized or localized, and mild to extensive erythema. A disturbed epidermal barrier function is often seen, which may lead to secondary signs such as increased trans-epidermal water loss and imbalances in nutrition but also severe eczema and allergies.
To investigate the epidermal barrier function and to assess novel therapeutics we have developed 3D full-thickness human skin models that mimic congenital ichthyosis, a rare and severe keratinization disorder. The model consists of an underlying dermal equivalent and a fully stratified, perfectly organized epidermal part with a well established basement membrane zone and all keratinocyte layers. The model has been validated using reference substances for reconstructed skin. We have employed the model for the characterization of an autosomal recessive skin disorder, the peeling skin disease. It is characterized by lifelong patchy peeling of the skin and severe erythroderma, associated with pruritus and atopy. The analysis of the epidermal permeation in models generated with patient keratinocytes clearly demonstrated an impairment of the epidermal barrier function, which is supposed to underlie the atopic phenotype in the patients. The models are now being used to investigate the abilities, effects, and toxicity of new drugs for therapeutic approaches specifically designed for genetic cornification disorders. The enhancement of epidermal penetration using nanoparticles was assessed in the in-vitro models with a model dye loaded onto the particles. Our animal-free model is the first human artificial skin model suitable for the characterization of epidermal barrier functional defects caused by monogenic disorders. It shows a high reproducibility of barrier properties and can be used more generally for the analysis of drugs as well as toxic substances.

F6-2

IN VITRO WOUND HEALING MODELS

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To monitor the healing process of wounded skin several *in vitro* methods are already described in the literature. Hereby, wounds were induced by deep scratching using a mesh or scalpel, punch biopsies or freeze damage. However, these procedures appear problematic with respect to reproducible wound sizes. To overcome this problem a new *in vitro* model for the investigation of wound healing has been established using a reconstructed full thickness skin model and a laser wounding procedure. Furthermore, its suitability for monitoring wound healing process was determined by testing the new approach of accelerating wound healing by the means of topically applied opioids.
The laser wounds were induced with a CO_2-Laser aiming for an almost complete destruction of irradiated epidermis without morphological alterations of the dermis and well defined wound margins. After method establishment healing process was investigated applying morphine solutions or morphine loaded nanoparticulate carrier systems onto the centre of the wounds. After a healing period of 4 days, skin models were analyzed using haematoxylin-eosin staining procedure.
Histological evaluation of the healing process revealed that morphine induced keratinocyte proliferation and migration from the margins and from the few remaining viable keratinocytes on the wound ground. Subsequently the wound ground was almost completely covered by a new epithelium which was not the case for the negative control. Moreover, semi-quantification of the thickness of the regenerated epidermis revealed a significantly thicker epidermis in those wounds treated with morphine solution compared to the control. Thus, morphine effects in the standardized laser-wounds are well in accordance with other publications showing the acceleration of wound healing by the means of topically applied opioids.

F6-4

IN VITRO INFECTION MODELS OF LOCALIZED CANDIDA INFECTIONS

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Basic research on the biology and immunology of microbial infection requires appropriate model systems. Due to the complexity of the processes, most studies involve animal testing. Besides ethical concerns, these models are not always representative of infections in humans, which holds true particularly for the human pathogenic fungus *Candida albicans*. In vitro models that closely parallel the in vivo situation and allow studies of relevant physiologic functions are thus highly desirable.
Possible alternatives, especially for localized infections, are provided by models using in vitro reconstituted human epithelium or epidermis. In recent years, these model systems have been successfully established to evaluate the effectiveness of topical anti-infectives, to characterize the role of fungal virulence factors, and to study the immune responses during localized *C. albicans* infections. Early studies focused on the consequence of gene disruption in *C. albicans* on pathogenicity and the epithelial cytokine pattern. Most recently, these models have been supplemented with immune cells such as lymphocytes and polymorphonuclear leukocytes to study their role during the course of infection and to characterize the interaction between the skin barrier and accessory immune cells. Using the in vitro model, it has been demonstrated that an immunological crosstalk between *C. albicans*–infected oral epithelium and polymorphonuclear leukocytes induced an immune cell-mediated upregulation of epithelial Toll like receptor 4, a member of an important receptor family which plays a critical role in innate immune recognition of pathogens. The increased receptor expression was directly responsible for protecting the mucosal surface from fungal invasion and cell injury. These studies will help us to get insights into the complex mechanisms by which appropriate innate and acquired immune responses are initiated and to identify factors that contribute to an increased susceptibility to *Candida* infection in patients.
Although any conclusion from these models for an in vivo infection has to be made with caution, the available systems reflect more and more the physiological situation found in vivo, thereby providing a valid matrix to model the events under controlled experimental conditions. In addition, such model systems can also be used to study infections with other fungi or bacteria.

F8-1

NEUE ERKENNTNISSE ZUR MOLEKULAREN UND MORPHOLOGISCHEN STRUKTUR DES STRATUM CORNEUM

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Im Vortrag werden zunächst die morphologischen Strukturelemente des Stratum corneum (SC), die Desmosomen und die „hakenähnlichen" Strukturen der Corneocyten vorgestellt, die den Raum für die hochgeordneten SC-Lipide schaffen. Danach werden neue Ergebnisse hinsichtlich des Einflusses der einzelnen SC-Lipide auf die molekulare Architektur der SC- Bilayer gezeigt. Die Resultate wurden mit der Neutronenstreuung erhalten.
Im Mittelpunkt dabei stehen die Ceramide und es wird der Einfluss der kurzkettigen Ceramide CER [AP] und CER [NP] den Aufbau der SC-Doppelschichten präsentiert. Es wird zunächst der Einfluss des hydrophilsten Ceramids des CER [AP] auf die Barrierefunktion des SC vorgestellt und gezeigt, dass dieses Ceramid essentiell für die Barrierefunktion des SC zu sein scheint.
Neue Ergebnisse der Arbeitsgruppe von Lars Norlen zeigen, dass im SC asymmetrische Doppelschichten vorliegen, da die kurzkettigen Ceramide unterschiedlich lange Alkylketten aufweisen. Die asymmetrischen Doppelschichten bestehen aus einer 45 nm-Doppelschicht, in der sich hauptsächlich Cholesterolmoleküle befinden, und einer 65 nm-Doppelschicht, in der sich die freien Fettsäuren befinden. Dabei scheint das CER [NP] in der gestreckten Konformation eine Schlüsselrolle zu spielen. Die Resultate von Norlen konnten unter Verwendung von deuterierter Lignocerinsäure und deuteriertem Cholesterol mit Neutronenstreuung bestätigt werden.
Außerdem wird gezeigt, wie sich Moleküle, die Penetration erhöhen (Enhancer), wie zum Beispiel Ölsäure und flüssige, synthetische Wachse, in die Doppelschichten der SC-Lipide einordnen.

F8-2

IN-VITRO METHODS TO DETERMINE THE DERMAL ABSORPTION. WHAT THEY CAN – WHERE ARE THEIR LIMITS?

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In-vitro methods to determine dermal absorption play an important role in product development in the field of pharmaceutics as well as cosmetics. In addition there is an increasing demand of such methods with respect to safety data of chemicals as recommended by the EU REACH program. In principle two different types of methods are used: Penetration models address the distribution of substances under investigation within the different skin layers. Whereas permeation models determine the diffusion of substances through the skin or selected skin layers (1). Different experimental setups for penetration studies will be presented and especially the different methods of skin segmentation will be shown. Moreover, possibilities of errors concerning penetration experiments will be displayed and their influence on the results will be discussed. As prototype for permeation experiments the well known Franz diffusion cell will be presented and the impact of the membrane used, e.g. full thickness skin, heat separated epidermis and bio-engineered skin constructs, on the results will be reviewed. In summary for both experimental set-ups the calculated parameters will be discussed concerning their comparability and the level of predictability.

(1) Hahn T., Schäfer U.F., Lehr C-M., SOFW-Journal, **136**, 28-40 (2010)

F8-3

PENETRATION AND STORAGE OF NANOPARTICLES IN THE SKIN

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The requirements on nanoparticles in cosmetics and medicine are very different in most cases. One the one hand, nanoparticles such as TiO_2 and ZnO, which are widely applied in sunscreens, should be localised on the skin surface in the upper cell layers of the stratum corneum, while during drug delivery they should penetrate the skin barrier in order to reach the target structures in living cells. The Charité utilises various methods to investigate the penetration and storage of nanoparticles in the skin, with hair follicles being in the focus of attention. Ideally suited as target structures for drug delivery, hair follicles are surrounded by a dense network of blood vessels and characterized by a high concentration of both stem and dendritic cells.

Investigations of nanoparticles of different size and materials showed that particles of circa 600 nm in diameter penetrate into the hair follicles particularly efficiently and can be stored there for up to 10 days. Thus, the retention time in the hair follicle is almost one order of magnitude longer than in the stratum corneum. The excellent penetration of these particles is due to the surface structure of the skin. The dandruff has a mean thickness of circa 600 nm and forms a “zigzag” structure on the hair surface. Obviously, this makes the moving hair acting like some sort of a gear pump and stimulates the transport process. The investigations did not show, however, that any particles with diameters between 40 nm and 1µm penetrated from the hair follicle into living tissue if the barrier was intact. This is plausible as the hair follicle, too, has a barrier structure of its own. Only in the event of artificial barrier damage a penetration of nanoparticles of 40 nm in diameter into living tissue structures could be observed. Consequently, a penetration through the intact skin barrier can be excluded for the investigated particle systems. But nanoparticles are also well suited to delivering drugs into the hair follicles for subsequent release.

All in all a toxicological evaluation of nanoparticles must primarily be made with respect to their chemical composition. Only secondarily it is to be examined if the specific structure of the systems leads to new, possibly hazardous properties.

F8-4

DERMATOLOGICAL VEHICLES – CLASSICAL AND INNOVATIVE FORMULATION CONCEPTS

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The efficacy, tolerability, and application properties of dermatological products are clearly related to the type of base used. Interactions between the vehicle, the skin, and the drug affect the rate of release of the active moiety and the therapeutic activity of the drug product.
The so called vehicle effect may be only a simple physical effect, e.g. cooling. In dependence on their composition, dermatological vehicles can, moreover, intensively interact with the skin and thus affect either positively or negatively the skin barrier function.
On diseased skin the acuity of the disease determines predominantly which type of vehicle should be used. Exceptions occur when special conditions of the site of application have to be considered, e.g. hairy skin regions.
To meet these multifaceted needs, a surprisingly large number of different vehicles are used in dermal therapy. Consequently, most dermatological drug substances, e.g. corticosteroids, are commercially available in different hydrophilic and lipophilic vehicles. If this is not the case, extemporaneous prescriptions may fill an existing therapeutic gap.
All this is necessary because the proper choice of the vehicle, whether it is hydrophilic or lipophilic, as well as its water, lipid, and emulsifier content allows to individualize and optimize dermal treatment.
The majority of products make use of classical dermatological vehicles, e.g. hydrophilic ointment or wool alcohol ointment, and variations thereof.
However, innovative formulation concepts with improved efficiency and tolerability are beginning to emerge. Frequently, the development of a new vehicle aims to enhance drug penetration. Moreover, cosmetic and usage criteria, e.g. absorption, spreadability, and skin feel, influence increasingly the development of new products. Although these parameters are primarily not responsible for the effectiveness of a drug product they largely affect patient compliance during long term therapy. Betulin stabilized emulsions (Betulsions) represent solid stabilized w/o emulsions which require an absolute minimum of ingredients because the active moiety acts simultaneously as a stabilizer. Foams are not only cosmetically elegant formulations they also allow for an almost touchless application, which might be extremely favorable for wound treatment.

F8-5

FORMULATION FOR THE TREATMENT OF SOLAR DAMAGES

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Solar damages resulting from insufficient protection against UV irradiation during the individual’s lifetime are expressed as actinic keratosis or even worse basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). One therapy option is the photodynamic therapy (PDT) involving three key components: a photosensitizer, light of an appropriate wavelength and oxygen within the tissue. As a photosensitizer precursor 5-aminolevulinic acid (ALA) is applied on the skin, has to be taken up by the cells and converted into protoporphyrin IX as the active photosensitizer. Since ALA permeation through the skin is slow and rather incomplete due to ALA’s hydrophilic properties, light exposure needs several hours subsequent to ALA application before being performed. The challenge was to develop a semisolid formulation with sufficient and fast permeation across the lipophilic barrier of the skin, the stratum corneum.
The developed semisolid liquid crystalline formulation consists of poloxamer 407, dimethyl isosorbide, isopropylic alcohol, propylene glycol dicaprate/dicaprylate and water. A predominantly higher permeation coefficient of 5-ALA across human SC was obtained from this formulation when compared to commonly used bases from the German Pharmacopoeia, such as Basiscreme DAC (increase by factor 7.4) and water containing hydrophilic ointment (increase by factor 19.4). Further permeation studies revealed a synergistic effect of all components leading to more than an additive effect on 5-ALA permeation enhancement.
Rheological measurements showed a reversible gelification upon heating. At refrigerator temperature the system was in the liquid state, at 12.7 °C it became a gel.
By means of differential calorimetry (DSC) strong interactions between the formulation and stratum corneum lipid structure could be detected. The results agree with the findings of the permeation results and explain an increased permeability of the stratum corneum. This increase in permeability would allow for a reduction of drug concentration and a shorter application - light exposure interval offering better compliance of the patients.

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